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A revolution by recombinant DNA technology to improve the quality of life

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Abstract

The emergence of recombinant DNA (rDNA) technology occurred through the appropriate use of known procedures and tools in novel ways that resulted in broad applications for modifying and analyzing gene structure and organization of complex genomes. In the past centuries, the production of organisms having desirable traits was a mere imagination. Nowadays, rDNA technology has revolutionized the field of science, having vast and multidisciplinary applications, and products that were impossible to produce by conventional or traditional methods. With the aid of rDNA technology, impossible things could be achieved. rDNA technology has vital applications like plant and animal production, health improvement, increment in food resources, treatment of serious diseases, protein development, improved environmental conditions, etc. This technology plays a crucial role not only in the betterment of health conditions by the development of pharmaceuticals or new vaccines but also in improving the treatment strategies by developing monitoring devices, novel therapeutic approaches, and new diagnostic kits. This review mainly emphasizes the possible roles of rDNA technology for human welfare.

Introduction

With the increase in human populations throughout the world, human life has become threatened by different factors, for example, malnutrition due to limited food, various types of contagious infections, atmospheric issues (resulting from the dramatic industrialization), and many more [1]. Despite the several methods that have been used so far, food yield is still much less than the actual requirement. There is a need for modern technologies to resolve these issues. Unlike traditional approaches, genetic engineering involves the latest techniques, like molecular cloning and transformation, that yield more reliable products by consuming less time [1]. For example, in comparison with traditional breeding, which transmits a huge number of both non-specific and specific genes to the recipient, Recombinant DNA (rDNA) technology transmits a few desired genes to the target by using a variety of approaches like Agrobacterium mediated transformation and biolistics [2]. Oligonucleotide-directed mutagenesis and recombinase mediated site specific genome insertion can also be employed [3].

rDNA technology started after the production of first rDNA by Paul Berg in 1970. For which he was awarded the Nobel Prize in 1980. First, genetically modified bacterium (*E. coli*) was created by Herbert Boyer [4]. Nowadays, a great number of genes are being cloned by using rDNA technology. Cloning refers to the formation of genetically identical individuals- asexually- for conserving the genetic characters [5]. Cloning can also be achieved through embryo spitting by shifting up to four single blastomeres from a 4-cell embryo into four different recipient mothers. In comparison, biotechnological approaches that rearrange, remove or add DNA to change phenotypes are called genetic engineering [5]. The application of genetic engineering in the creation of genetically modified organisms is the most advanced development contributing to the fields of agriculture, medicine, industries, bioremediation, and many more [6]. Organism genomes can be edited by introducing novel genes and regulatory elements. This can also be achieved by the recombination of elements and genes to reduce or obstruct the expression of endogenous genes [7].

According to the Food and Drug Administration (FDA), meat and milk from cloned species are safe for consumption [8]. Biotechnologies; including cloning, genetic engineering and gene editing, have been also used for the preparation of vaccines, and microbial fermentation of feedstuffs [5]. Moreover, gene therapy has emerged as the mean of selective integration of pathogen-resistance genes into the human genome. This strategy has the potential to improve public health dramatically and to reduce the use of drugs. This would herald an age of personalized medical care, with ramifications for the pharmaceutical industry [6].

rDNA technology strategies have been also employed for bioremediation, biomining, and the production of biofuels and bioethanol [9-12]. Thus, in the 21st century, rDNA technology has an enhancing effect on every aspect of our life. This review defines the challenges faced by humans and their solutions via rDNA technology.

Role of rDNA technology in Biotechnology

The term "biotechnology" indicates the use of living systems or molecular engineering to form products for patient care and to launch biologic therapies [13]. The biotechnology industry was dominated by genetic engineering or rDNA technology for more than a decade. It was aimed to enhance the standards of human life by employing its techniques in agricultural communities, and was a part of cheese, bread and beverages production, and product preservation. But today's biotechnology is different from that of past, as it has a variety of implications in every aspect of life [14].

The technique involving the *in vitro* combination of DNA molecules of different origins resulting in the organism of desirable traits is known as rDNA technology or genetic engineering [15]. The detailed procedure of rDNA technology has been described in **Fig. 1**. Briefly, rDNA is produced by combining at least two different strands of DNA extracted from any species, also called sometimes chimeric DNA. For instance, human DNA can be combined with bacterial DNA or plant DNA can be combined with fungal DNA. By using rDNA technology, any sequence of DNA can be produced and introduced into any living organism, *i.e.*, bacteria, yeasts, etc. to produce that gene in volume. This requires a cloning vector (derived from viruses or plasmids) and a DNA molecule that is going to replicate within a living cell [16].

After the discovery of restriction endonucleases in 1978, the interest in the rDNA was considerably heightened [17]. This enzyme cuts the DNA molecule at a certain point, then the foreign DNA combines at this point. DNA ligase enzyme then joins the two molecules of DNA permanently. In this way, by

utilizing biotechnology tools, scientists can change the nature of DNA, and subsequently, the genetic makeup of organisms can be enhanced. The medical benefits of genetic manipulations have pioneered a new trend in biotechnology for molecular cloning methods to transmit genes expressing desirable characters into another host organism, thereby producing favorable phenotypes [6]. During the last century, there has been extensive
research on microorganisms that made the rDNA technology a reality. *Escherichia coli* is the most important organism in rDNA research. Scientists are now developing new medicines, high yielding and nutrients rich crops, new vaccines, environment-friendly alternatives to plastics and fossil fuels, etc., with the aid of rDNA technology. Nowadays, many recombinants products are available at the market like human insulin, interferons, growth, hormones, etc. that are created by the rDNA technology [18]. Gene cloning is one of the widely applied tools in researches related to proteins and rDNA technology. Gene cloning involves the cleavage of plasmid and inserting the sequence into it with the help of restriction enzymes, and at the last, joining them together permanently by ligase enzyme. This followed by its insertion into a simple organism, allowing its replication. These also carry genes imparting useful properties to the host, like toxin production, antibiotic resistance, etc. This strategy is much convenient and straightforward in the case of well-positioned restriction sites in the sequences but becomes problematic in the absence of such restriction sites [19].

Genetic manipulations have taken the place of traditional techniques and have the huge potential to defeat such challenges [1]. There are applications of rDNA technology in almost every field from the medical industry to the food industry [20].

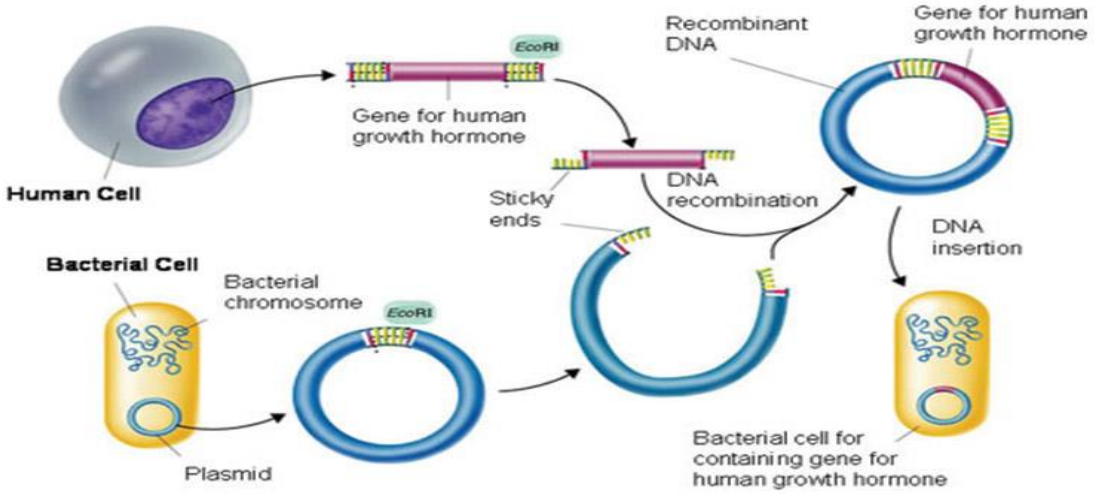

Fig. 1. The procedure of rDNA technology.
Firstly, the plasmid and gene of interest are isolated from bacterial cell and human cell respectively. Both are restricted using the similar enzyme. The gene of interest is then joined with sticky ends of plasmid, resulting in the formation of rDNA which is ready for the insertion into the target cell.
Adapted from Microbe Notes by Sagar Aryal, 2018, Retrieved from: https://microbenotes.com/wp-content/uploads/2018/09/Recombinant-DNA-technology.jpg

Genetically Modified Organisms (GMOs)

Animals whose genome has been modified by rDNA technology, either by the insertion of foreign DNA or transfer of gene/genes to achieve the desirable traits are called transgenic animals or Genetically Modified Organisms (GMOs) [21]. The rDNA has been created in a laboratory to insert it into the genome of animals. Transgenic animals e.g., genetically modified mice, rodents are used in laboratories routinely as models in biomedical research. They are used to study the progression of diseases, researching human diseases, responses to therapeutic interventions [22]. Genetically modified mice are also being used to produce human Abs to be used as therapeutics [23].
Transgenic animals are also used to prepare complex human proteins at large scale that are used for treating various diseases in humans. But the production of

these therapeutic proteins is an expensive process. The low-cost alternative is to produce these recombinant proteins in the blood, eggs, or milk of genetically modified animals. Up till now, only two recombinant products have been approved by the FDA. The first one is human antithrombin III, which is used to avoid clots in patients having antithrombin deficiency, this therapeutic protein was produced in the milk of transgenic goats [24]. Almost 80 transgenic goats are enough to supply antithrombin III to all of Europe. The second one is C12 esterase inhibitor that is used to treat very rare genetic disorders i.e. hereditary angioedema that causes blood vessels to expand and result in skin swellings. This protein was produced in the milk of transgenic rabbits[25].

Transgenic animals also play many other critical roles. For example, mice models can be used for learning human diseases with CRISPR, where the study of individual genes becomes easier [26]. Nowadays, the study of gene interactions has also become possible by altering multiple genes in cells [27]. For example, transgenic rodents are developed to understand human diseases because of similarities in gene function and physiology among humans and rodents. Transgenic mice are used to study heart diseases, obesity, anxiety, diabetes, aging, arthritis, Parkinson's disease, cancer, and many more [28-30]. This is all possible because of rDNA technology. Today many transgenic animals have been produced (Goat, Cow, Mice, Rabbits, Pigs, Sheep, fluorescent Zebrafish, Glofish, Catfish, Goldfish, Salmon etc). some of these are mentioned in **Table 1**.

Table 1: Examples of transgenic animals

Species	Transgene or target phenotype	Consumer Benefit	References
Bovine	Bovine beta casein	Enhanced expression of casein proteins	[31]
Carp	Growth hormone	Enhanced growth rate	[32]
Sheep	Alpha-1 Antitrypsin	High level expression of human alpha-1-antitrypsin in milk	[33]
Sheep	Keratin Associated protein gene	Novel wool traits	[34]
Pacific oyster (Crassostrea grass)	Fluorescent protein	Tool for basic genetic studies	[35]
Atlantic salmon	Freeze resistant	Extended range of sites to grow	[32]

Human insulin from rDNA technology

Insulin is a well-characterized endocrine peptide hormone which plays an essential role in supplying energy to body cells [36]. This hormone indirectly or directly affects every tissue or organ in the body. Its main function is to activate anabolic reactions for fats, proteins, and carbohydrates resulting in a reduced blood glucose level. Its deficiency in the body results in the disease diabetes mellitus [37].

Initially, the insulin from animal sources was capable of treating diabetic patients, these initial preparations had highly variable efficacy. Impurities in animal-based insulins resulted in the side effects including lipoatrophy and insulin allergy. This prompted the latest researchers to innovate procedures for insulin purification. Therefore, recombinant and synthetic human insulin products were created to improve insulin purity as well as reproducibility of response [38]. Human insulin developed by rDNA technique is the first commercial health care product derived from this technology [37, 39]. This insulin was very helpful in extending the life spans of diabetic patients who otherwise would have slowly died because of glucose unavailability to body cells [37]. Before the discovery of rDNA technology, insulin was usually extracted from the pancreas of pigs and cows that resulted in an allergic reaction in almost 5% of patients because the structure of cow's or pig's insulin was different from that of human insulin. Whereas, genetically synthesized human insulin seemed to be safe and effective in man [40]. By using rDNA technology, *E. coli* is being utilized to produce different types of hormones, *i.e.*, somatostatin, p-endorphin, somatotropin, etc. These hormones are produced in fair amounts and have commercial importance [41, 42].

The detailed procedure for the genetic synthesis of insulin is given in Fig 2. Briefly, artificially prepared genes for human insulin A and B chains are cloned separately in a suitable vector like pBR322 plasmid. The cloned synthetic genes are then integrated with a beta-galactosidase gene of *E. coli* to provide well-organized transcription and translation, and a stable precursor protein. The insulin peptides are purified after cleavage from a beta-galactosidase gene. Complete purification of A chain and partial purification of B chain is carried out. These products are then mixed, reduced, and re-oxidized. Radioimmunoassay is used to detect the presence of insulin [43].

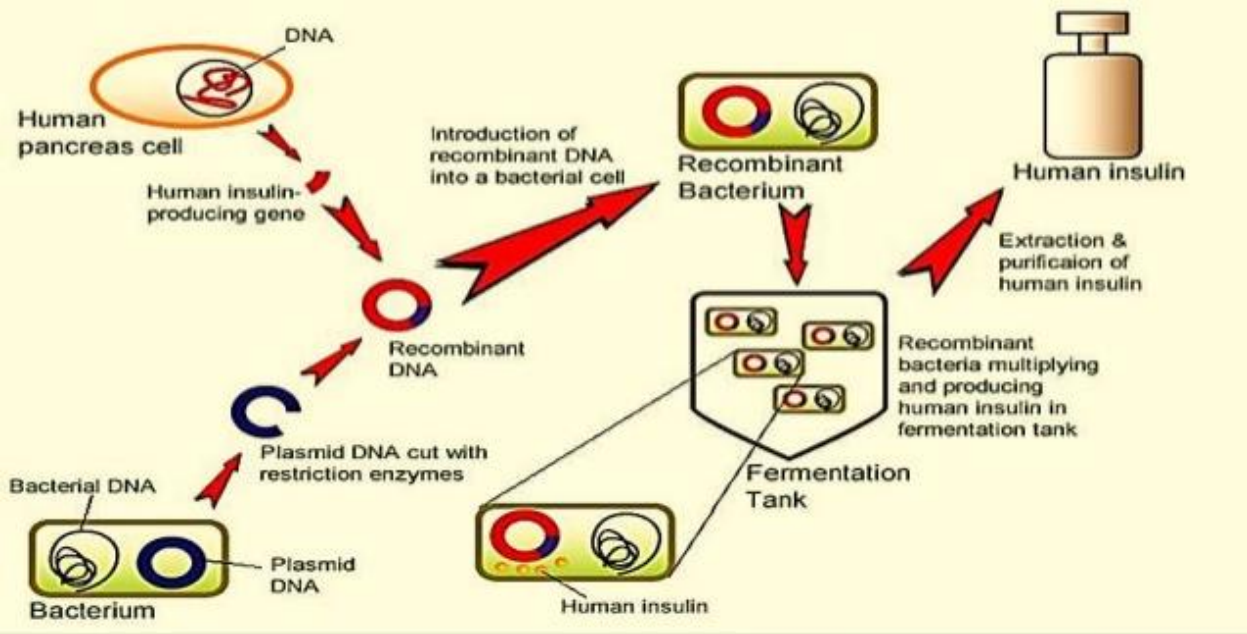

Fig. 2: Production of human insulin by rDNA technology.
Adapted from slide share by Nancy Saber, Roba Shaat and Mohamed El-Asaly, 2017, Retrieved from https://image.slidesharecdn.com/insulinpresentation-170512235659/95/insulin-production-and-synthesis-19-638.jpg?cb=1494633749

Vaccine production

The chemical preparations that contain weakened or inactive pathogen, injected to animals or human beings to produce immunity to a certain disease, is known as vaccine. A potential vaccine induces the production of viral specific antibodies that neutralize viruses and prevent infection [44, 45]. The production of vaccines is another important application of rDNA technology[46]. Before the discovery of rDNA technology, vaccines were produced by using entire pathogens that made it dangerous. However, nowadays vaccines are prepared by using the desired antigen, as an antigen will not be able to replicate so there will be no induction of disease [47].

Recombinant vaccines are more specific and efficient as compared to conventional vaccines [48]. The genes coding for antigen are transferred to the disease producing bacteria. These neutralizing antibodies then protect against the same bacteria or virus that caused infection. rDNA vaccine has revolutionized the treatment strategies of infectious diseases. DNA vaccines contain a gene from the concerned pathogen that encodes immunogenic protein [49].

rDNA vaccines have long shelf lives. Today, a huge number of vaccines are being synthesized through rDNA technology. These may include vaccines for hepatitis, polio, cholera, malaria, foot and mouth disease, etc. that were impossible without rDNA technology [50-53]. To give an example, hepatitis B vaccine is comprised of viral proteins that are secreted by transgenic yeast cells containing viral genes. This recombinant vaccine is safely used due to the absence of viral particles in it. Acquired immune deficiency syndrome (AIDS) vaccine is being produced experimentally in the same way [47].

Production of Monoclonal antibodies

Monoclonal antibodies (mAbs) exhibit a wide range of biological activities with high specificity. Paul Ehrlich hypothesized mAbs as magic bullets because of their specific ligand-binding activity [54]. mAbs have a high potential in the development of bioactive peptides and diagnostics [55]. These are efficient in treating cancer, so these are being produced for cancer treatment [56]. There are five families or isotopes of antibodies in humans e.g., immunoglobulin alpha (IgA), immunoglobulin epsilon (IgE), immunoglobulin delta (IgD), immunoglobulin mu (IgM), and immunoglobulin gamma (IgG). Out of these, IgG is the most prevalent with highest therapeutic ability [57]. Many mAbs are being produced on large scale [58]. Between 2006 and 2011, seven different antibodies were derived from the transgenic mice, these monoclonal antibodies were

also approved by the FDA [59]. Today almost 20 mAbs are approved for human use. mAbs were produced *in vitro* by murine hybridoma technology, developed by Milstein and Kohler, in which there was fusion of B-cells and myeloma cells [60]. With the development of this technique, the capability to prepare huge amounts of well-characterized monoclonal antibodies has revolutionized the therapeutic medicine and diagnostics [61]. This technology was not very helpful because many antibodies that were derived from murine cells showed the problem of low immunogenicity [19]. In near future, it is expected that more mAbs will be available in the market because of rDNA technology. A major limitation of using mAbs is that these are recognized as foreign invaders by the patient, resulting in an antiglobulin reaction. rDNA technology offers the possibility for the conversion of rodent based antibodies into a human form. At the gene level, it is possible to subclone the rodent exons encoding the variable regions and the human exons encoding the constant regions and then to recombine these segments to form a new chimeric gene. When recombined with a suitable expression vector and transformed into appropriate cells, the exons are spliced together to give rise to a contiguous mRNA that encodes for a chimeric protein molecule [62].

Production of interferons

The virus induced proteins that are produced by the viral infected cells are known as interferons [63]. These are glycoproteins in nature [64]. Interferons are antiviral in action but they also have anti-cancerous property [65]. Thus, these act as the first line of defense against serious infections caused by viruses, such as lymph nodes malignancy and breast cancer [66]. Currently, interferons are used against certain skin conditions and certain types of cancers [67].

Naturally, interferons are produced in minute quantities in human blood and their *in vitro* production is much costly. These can also be produced using a cheaper method i.e., rDNA technology. For that purpose, the genes of the fibroblast from humans (that produce interferons) are isolated and inserted into the plasmid of bacteria. These modified bacteria then produce clones that secrete interferons in large quantities. Interferons then produced are extracted and purified from bacteria [68]. Randomized, double-blind and placebo-controlled trials concluded the beneficial effects of recombinant interferon alpha in reducing disease activity in chronic hepatitis C [69, 70]. A therapy involving subcutaneous inoculation of recombinant interferon gamma has been proved effective and safe in the treatment of atopic dermatitis [71]. Recombinant interferons may also help in host defense against *Salmonella* and *Shigella* infections [72].

Production of Antibiotics

Antibiotics are produced on large scale using fermentation processes [73]. The enhancement of the industrial production of antibiotics has been achieved by the means of rDNA technology [74]. The antibiotic-producing microbial strains are now manipulated through genetic modifications for improving the large-scale production of antibiotics [18]. Strain enhancement procedures allow the blockage of unnecessary enzyme functions, increase in gene dosage, removal of negative regulations and many more. Strains can be genetically modified to increase the antibiotic production, fermentation at different temperatures, use of cheaper raw material, short fermentation batches or decreased oxygen needs, etc. [74, 75]. The initiation of rDNA approaches and their application to antibiotic-secreting microbes has also allowed the design of biosynthetic pathways giving rise to novel antibiotics. For example, genetic manipulation of *Cephalosporium acremonium* -cephalosporin producing fungus- has resulted in yield improvements, increased biosynthetic dosage, enhanced oxygen utilization, and new biosynthetic capacities by 7-aminocephalosporanic acid (7-ACA) or penicillin G production [76]. Similarly, in *Penicillium chrysogenum* -the industrial penicillin producing fungus-heterologous expression of cephalosporin biosynthetic genes has led to the secretion of adipyl-7-aminodeacetoxycephalosporanic acid (adipyl-7-ADCA) and adipyl-7-ACA, compounds that can be transformed into the economically relevant 7-ADCA and 7-ACA intermediates [77]. Likewise, the expression of genes encoding cephalosporin acylase and D-amino acid oxidase activities in *Escherichia coli* has simplified the bioconversion of cephalosporin C into 7-ACA, diminishing the uptake of organic solvents [78]. Likewise, the genetic modifications in actinomycetes -an antibiotic producer- have resulted in increased yields and the production of new hybrid antibiotics [74]. Antibiotics that are produced by genetically modified organisms are extremely effective against viral, protozoan, and bacterial diseases [75].

Gene therapy

Humans are affected by many genetic disorders caused by a mutation in one or more genes, resulting in the change of DNA sequence. Genetic disorders are either inherited from parents like sickle cell disease, or due to environmental exposure (cigarette smoke) like neurofibromatosis and cancer. Genetic disorders have taken the lives of millions of people due to the lack of proper treatment. They are emerging at an alarming rate especially in developing countries [79]. rDNA technology has now made the treatment of genetic disorders possible by a process known as gene therapy [80]. It is a rDNA technology process, in this technique, defective genes taken from patients, modified by adding or removing genes, and then replaced with normal genes in patients. This normal gene allows the synthesis of protein that was lacking before [81].

It is used to treat different types of cancers including neurological, lung, skin, gastrointestinal tumors, gynecological, pediatric tumors, urological and hematological malignancies [80, 82]. In the treatment of skin cancer (melanoma) lymphocytes that attack the tumor cells are taken from patients and they are treated with genes having anticancer proteins known as tumor necrosis factor. These modified lymphocytes then reinfused into patients; these altered lymphocytes produce new protein that destroys the tumor cells. Insertion of tumor suppressor genes in cases of oncolytic virotherapy, gene-directed enzyme prodrug therapy and immunotherapy are different strategies that have been used to treat various kinds of cancers [1, 83].

In various cancer treatments, p53 (tumor suppressor gene) is commonly transferred [84]. The most significant approaches that have been utilized until now are vaccination with cancer cells engineered to express immunostimulatory molecules, vaccination with recombinant viral vectors encoding tumor antigens, and vaccination with host cells engineered to express tumor antigens [82]. Tumor cells can be destroyed by oncolytic viruses through viral replication and by arming with therapeutic transgenes. Recently, the efficacy of cancer gene therapy has been also improved [85].

Pharmaceutical Products

There is an increasing demand for the development of recombinant pharmaceutical proteins for a broad range of therapeutic applications [86]. Industrial enzymes and pharmaceuticals were the first biotechnology products on the world market that were prepared via rDNA technology [87]. Drugs developed by the aid of live organisms with the assistance of rDNA technology are known as biologics, biopharmaceuticals, rDNA expressed products, bioengineered, or genetically engineered drugs [82]. The pharmaceutical products synthesized through rDNA technique completely changed human life in such a way that the U.S. FDA approved more recombinant drugs in 1997 than in the previous several years, that are used for the treatment of AIDS, anemia, different cancers including leukemia, Kaposi's sarcoma, and ovarian, colorectal, and kidney cancers, diabetic foot ulcers, hepatitis (B and C), genital warts, diphtheria, multiple sclerosis, human growth hormone deficiency, and hereditary disorders like familial hypercholesterolemia, cystic fibrosis, hemophilia A, Gaucher's disease, Turner's syndrome, and severe combined immunodeficiency disease, [1].

Most pharmaceutical proteins have been produced in transgenic tobacco plants [88].The field of preparing recombinant pharmaceutical proteins using plants is entering a new phase with the recent approval of recombinant glucocerebrosidase secreted in carrot cells and the successful development of plant based clinical-grade proteins [89].

Nowadays, a plethora of blood factors, vaccines, growth hormones, biopharmaceuticals, enzymes, and therapeutic proteins have been synthesized on industrial scale [90]. Genetic manipulation of antitumor biosynthetic pathways will offer an alternative for the generation of novel anti-cancer drugs in the near future [91]. Different recombinant techniques to prepare lactoferrin have been developed. Human lactoferrin obtained from transgenic cows has met the criteria of high yield, structural similarity, and ease of protein purification [92]. Recombinant human erythropoietin -the main regulator of erythropoiesis- is commercially produced by the expression of erythropoietin complementary DNA clones in eukaryotic cell lines, most commonly in baby hamster kidney cells or Chinese hamster ovary cells [93].

Small molecules' drugs preparation via rDNA technology

Small molecules and proteins are the preferred modalities in drug development and major forms of medications that target protein including transporters, ion channels, receptors, kinases and enzymes [94]. Small molecules' drugs have shown promising results

in the treatment of COVID-19 [95-98]. These have been also prepared using rDNA technology [99].

Disease-modifying antirheumatic drugs (DMARDs), also known as "targeted biologic agents," "biologic agents," or simply "biologics," are produced by rDNA technology. These are a group of medications commonly used for treating rheumatoid arthritis [100]. These were designed to decrease or prevent inflammation that damage joint, and to reduce the effects of rheumatoid arthritis [101].

In previous researches, a series of chloramphenicol acetyl transferase (CAT) recombinants expressing CAT under the control of UL54 (DNA polymerase, POL) or UL99 (pp28) promoters were constructed. The secretion of CAT in infected cells highly mimicked the expression pattern of the endogenous UL54 and UL99. Thus, these two gene promoters were selected to construct luciferase-recombinant cytomegalovirus for quantification of CMV replication in a quick and reproducible way. The pp28-luciferase reporter system is rapid, highly sensitive and reproducible. It may be applied to screening of novel anti-CMV compounds [102]. The luciferase-based technique provides huge benefits over other existing procedures including its ease, rapidness, minimal equipment requirement, and relatively low cost [99].

rDNA technology in Agriculture

In agriculture, rDNA technology has vast applications. It is used in biotechnology to produce genetically modified plants (transgenic plants) to achieve insect-remittance, disease resistance, stress tolerance, higher yield, more nutrients, better quality, and more tastier crops. Genes are transferred to plants to achieve desirable traits like increased photosynthetic rate and increased starch production among others [103].

Among plants site-specific integration, multigene transfer and specifically regulated gene expression are the most recent strategies [104]. The first genetically engineered crop that was grown commercially and be granted a license for human consumption was the genetically modified tomato, after that many genetically modified crops were produced. For example, Golden rice is the most important example of transgenic plants made through rDNA technology. Golden rice has a high value of Vitamin A to cope with the vitamin deficiency. Similarly, plants are genetically engineered to become resistant to pests. Genes are taken from the bacterium *Bacillus thuringiensis* and then inserted into plants to make them resistant to different pests, e.g., BT corn etc. [105].

The yield and quality of crops are improving with the help of rDNA technology in developing countries. Biotechnology and rDNA technology are being used to enhance nitrogen fixation by plants. The genes of nitrogen fixation are obtained from the bacteria and inserted into plants. So, that plants can obtain nitrogen directly from the atmosphere and synthesize their proteins without the assistance of bacteria [106].

Many plants produce secondary metabolites having a variety of roles such as defense molecules against the attack by pests and pollinator attractants (e.g., scents and pigments). Secondary metabolites are sources of fragrances, food additives, pharmaceuticals, agrochemicals, etc. As most of the secondary metabolites are originated from plants, any climatic factor will endanger the world supply. Nevertheless, the yields obtained from cultured cells are often inferior to the amounts present in intact plants, provided a major drawback to their commercial exploitation [107]. Almost 200 species of plants have been genetically modified successfully over the last 10-15 years. After that, attempts have been made on the genetic transformation of plants to increase the production of pigments, flavors, and also various pharmaceuticals [108]. The key enzymes and intermediates participating in the expression of secondary metabolites can be interpreted by the turned cultures [109]. Some of the examples of previously genetically modified plants have been listed in **Table 2** [110].

rDNA technology in food industries

rDNA technology has applications in food-related areas like the production of enzymes, processing of cheese, beverages, agricultural raw materials, etc. [132]. It is used for the production of several enzymes suitable for food processing. Several enzymes have particular applications and roles in the food industry like amylases and lipases. Enhancement of microbial strains for the increased yield of enzymes by rDNA technology is a huge achievement [133]. In recent few years, there has been growing interest in modifying lysozyme in order to enhance its activity against Gram-negative bacteria [134]. Recombinant lysozymes are widely used in food industries for inhibiting bacterial growth causing contaminations [135]. They are used to promote the shelf life of meat, vegetables, fruits, and cheese. This is achieved by the immobilization of lysozymes in the cellulose. Lysozyme impregnation of fish skin gelatin gels

Table 2: Genetically modified plants.

Crops	Target genes	Traits	References
Canola	cp4 epsps and gox genes	Glyphosate tolerance	[111]
	pat and nptII genes	Glufosinate ammonium tolerance	[112]
	Barnase and barstar genes	Pollination control and glufosinate ammonium tolerance bar genes	[113]
	Nitrilase gene	Tolerance to oxynil herbicides like ioxynil and bromoxynil	[114]
	NptII gene	High laurate	[115]
Corn	Epsps gene	Glyphosate tolerance	[116]
	Synthetic pat and bla genes	Glufosinate ammonium tolerance	[117]
	Cry1Ab gene	Corn rootworm resistance	[118]
Soybean	cp4 epsps and gox genes	Glyphosate tolerance	[111]
	pat gene	Glufosinate ammonium tolerance	[119]
	fad2 gene	High oleic acid soybean oil	[120]
	gus gene	Fatty acid desaturase	[121]
Cotton	nptII and Cp4 epsps genes	Glyphosate tolerance	[122]
	nptII and bxn genes	Resistance against oxynil herbicides like ioxynil and bromoxynil	[123]
	Cry1Ac gene	Resistance to lepidopteran insects	[124]
Flax	ALS gene	Sulfonylurea herbicide tolerance	[125]
Papaya	CP gene	Tolerance against papaya ring spot virus	[126]
Potato	cry3A and nptII genes	Resistance to Colorado potato beetle	[127]
Squash	CMV/WMV2cp, ZYMVcp genes	Resistance against viral infection	[128]
Sugar beet	Bar and gus A genes	Phosphinothricin (PPT) herbicide tolerance	[129]
Tomato	pg (antisense polygalacturonase) and nptII genes	Delayed ripening	[130]
	cry1Ac gene	Resistance to Lepidopteran insects	[131]

increases the shelf life of food products and inhibit food spoilage [136-138]. Biofilms in the production area of food industries can be removed by the combining activity of amylases and serine proteases [139].

Glucose oxidase has been also manufactured by rDNA technology [140]. It is such a recombinant lysozyme which is useful in the inhibition of various food spoiling microbes including, *Salmonella infantis*, *Bacillus. cereus*, *Listeria. monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, *Campylobacter jejuni*, *Yersinia enterocolitica* etc. It is among the main enzymes used in food industry and kills a wide range of foodborne pathogens [138].

Contributions to enhanced environmental conditions

rDNA technology has many applications to solve environmental issues in various ways. Recent approaches in rDNA technology include bioremediation and waste treatment. Naturally occurring microbes are involved in environmental biotechnology for a wide range of applications in waste management. The bioremediation process, utilizing microbes, is currently in progress to clean up contaminated waterways, air, lakes and land [19]. Likewise, different microbes are used for the treatment of wastewater, sewage, industrial effluents, etc. [141]. Bioremediation makes use of microorganisms for environmental protection [142]. For example, *Nitrosomonas europaea* and *Pseudomonas putida* are being utilized in the bioremediation process [143, 144]. The incorporation of the marker genes is the simplest way to produce genetically modified antibiotic-resistant bacteria. This may affect normal ecosystems; for instance, bacteria having petroleum degrading ability cause destruction of imported petroleum products [6]. rDNA technology is also used in the production of bioindicators. These are bacteria that have been genetically modified as 'bioluminescent' - capable of emitting light in response to several chemical pollutants [19]. These bioindicator bacteria are used to estimate the environmental contaminations by toxic chemicals.

Environment-friendly fuels, obtained from biomass, are known as biofuels. They are cost-effective and renewable. rDNA technology is playing an essential part in the large-scale and beneficial production of the biofuels such as bioethanol, biodiesel, biohydrogen etc. Many microorganism's mediate hydrogen production especially cyanobacteria. But their production requires specific enzymes. rDNA technology has made possible higher product tolerance and product yield by improving the microorganisms [145].

These microorganisms are being created by using the modern rDNA technology, which results in the high production of bioenergy [146]. Unlike the conventional energy sources, bioenergy production does not release CO_2 or other hazardous chemicals. Thus, it helps to keep the environment safe and clean [107]. This strategy has proved successful for a wide range of commodity chemicals, mostly energy carriers, including medium chained and short-chained

alcohols [147]. Some energy crop plants are also used to produce biomass because they use solar energy in a better way. The microbes that are used in fermentation are also utilized for the production of biogas. This is all possible only because of rDNA technology.

Social impact of genetic manipulation

There are also some hazardous risks of genetic engineering [148-150]. For instance, the release of genetically modified species in the environment may destroy naive species or disturb the ecological equilibrium i.e., loss of biodiversity [151]. Hazardous toxins, produced by genetic modification of microorganisms, like aflatoxins, botulinum toxin, etc., can be used as biological weapons. Moreover, resilient plants may give rise to resilient weeds that would be hard to control [152]. People are worried about the safety of genetically modified foods and medicines. Some people are allergic to genetically modified food, so it causes health effects. There may be the risk of alteration in nutritional quality of genetically modified food [152]. Terrorists may produce dangerous biological warfare agents that may result in bioterrorism [153]. On the other hand, careless handling during the process may cause the release of recombinant organisms from the lab to the natural environment. That would be extremely dangerous. Moreover, the genetic insertion of a gene into the incorrect site may result in progeny with deformities. For example, the genetically altered seeds were suspected as the cause of 2012 epidemic that swept through commercial bee colonies. Approximately 50% of the nation's bee population was wiped out during the pandemic, with farmers in California being hit the hardest [154]. There is also a privacy issue. People are much concerned about their genetic information that could be used without their permission. There are also some ethical concerns as the scientists are trying to play with nature and messing with natural selection [155]. Thus, rDNA technology presents an exciting range of possibilities, from feeding the hungry to preventing and treating diseases; however, these promises are not without potential peril [150].

Conclusions

rDNA technology has made human life easier through important developments in science. This field has grown more than any other field in the last 15 years. In the recent decade, rDNA technology has evolved novel approaches for the biomedical field, such as diabetes treatment, genetic disorders, cancer, and many plant disorders especially fungal and viral infections. It also has several roles in agriculture. rDNA technology has brought significant improvements not only in humans but also in microorganisms and plants. There are some serious difficulties in the improvement of products at the gene level that should be solved for the future. Today, it is playing an essential part in the treatment of several diseases that were not possible before. It is providing benefits virtually to everyone in any profession in the possible way.

Conflict of interest

The authors declare no conflict of interest.

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