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Recombinant DNA Technology and its Applications: A Review

S. A. Shinde*, S. A. Chavhan, S. B.Sapkal, V. N. Shrikhande

Dr. Rajendra Gode College of Pharmacy, Malkapur Dist- Buldana(MS), India

Mob. No:-09890251512

Abstract: Biotechnology which is synonymous with genetic engineering or recombinant DNA (rDNA) is an industrial process that uses the scientific research on DNA for practical applications. rDNA is a form of artificial DNA that is made through the combination or insertion of one or more DNA strands,It offered new opportunities for innovations to produce a wide range of therapeutic products with immediate effect in the medical genetics and biomedicine by modifying microorganisms, animals, and plants to yield medically useful substances.Recombinant DNA technology is playing a vital role in improving health conditions by developing new vaccines and pharmaceuticals. This review gives brief introduction to rDNA and its applications in various fields.

Key words: Chimeric DNA, restriction enzymes, Transgenic Plants, Gene Therapy.

Introduction:

Human life is greatly affected by three factors: deficiency of food, health problems, and environmental issues. Food and health are basic human requirements beside a clean and safe environment. With increasing world's population at a greater rate, human requirements for food are rapidly increasing. Humans require safe-food at reasonable price. Several human related health issues across the globe cause large number of deaths. Approximately 36 million people die each year from noncommunicable and communicable diseases, such as cardiovascular diseases, cancer, diabetes, AIDS/HIV, tuberculosis, malaria. Despite extensive efforts being made, the current world food production is much lower than human requirements, and health facilities are even below standard in the third-world countries. Rapid increase in industrialization has soared up the environmental pollution and industrial wastes are directly allowed to mix with water, which has affected aquatic marines and, indirectly, human-beings. Therefore, these issues urge to be addressed through modern technologies.

Unlike tradition approaches to overcome agriculture, health, and environmental issues through breeding, traditional medicines, and pollutants degradation through conventional techniques respectively, the genetic engineering utilizes modern tools and approaches, such as molecular cloning and transformation, which are less time consuming and yield more reliable products. For example, compared to conventional breeding that transfers a large number of both specific and nonspecific genes to the recipient, genetic engineering only transfers a small block of desired genes to the target through various approaches, such as biolistic and Agrobacterium-mediated transformation [1]. The alteration into plant genomes is brought either by homologous

recombination dependent gene targeting or by nuclease-mediated site-specific genome modification. Recombinase mediated site-specific genome integration and oligonucleotide directed mutagenesis can also be used [3].

Recombinant DNA technology is playing a vital role in improving health conditions by developing new vaccines and pharmaceuticals. The treatment strategies are also improved by developing diagnostic kits, monitoring devices, and new therapeutic approaches. Synthesis of synthetic human insulin and erythropoietin by genetically modified bacteria [3] and production of new types of experimental mutant mice for research purposes are one of the leading examples of genetic engineering in health. Likewise, genetic engineering strategies have been employed to tackle the environmental issues such as converting wastes into biofuels and bioethanol [4-7], cleaning the oil spills, carbon, and other toxic wastes, and detecting arsenic and other contaminants in drinking water. The genetically modified microbes are also effectively used in biomining and bioremediation.

The advent of recombinant DNA technology revolutionized the development in biology and led to a series of dramatic changes. It offered new opportunities for innovations to produce a wide range of therapeutic products with immediate effect in the medical genetics and biomedicine by modifying microorganisms, animals, and plants to yield medically useful substances [8, 9]. Most biotechnology pharmaceuticals are recombinant in nature which plays a key role against human lethal diseases. The pharmaceutical products synthesized through recombinant DNA technology, completely changed the human life in such a way that the U.S. Food and Drug Administration (FDA) approved more recombinant drugs in 1997 than in the previous several years combined, which includes anaemia, AIDS, cancers (Kaposi's sarcoma, leukemia, and colorectal, kidney, and ovarian cancers), hereditary disorders (cystic fibrosis, familial hypercholesterolemia, Gaucher's disease, haemophilia A, severe combined immunodeficiency disease, and Turners' syndrome), diabetic foot ulcers, diphtheria, genital warts, hepatitis B, hepatitis C, human growth hormone deficiency, and multiple sclerosis. Considering the plants develop multigene transfer, site-specific integration and specifically regulated gene expression are crucial advanced approaches [10]. Transcriptional regulation of endogenous genes, their effectiveness in the new locations, and the precise control of transgene expression are major challenges in plant biotechnology which need further developments for them to be used successfully[11].

Recombinant DNA technology: A series of procedures that are used to join together (recombine) DNA segments. A recombinant DNA molecule is constructed from segments of two or more different DNA molecules. Under certain conditions, a recombinant DNA molecule can enter a cell and replicate there, either on its own or after it has been integrated into a chromosome.

Recombinant DNA (**rDNA**) molecules are DNA molecules formed by laboratory methods of genetic recombination (such as molecular cloning) to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in the genome. Recombinant DNA was first achieved in 1973 Herbert Boyer, of the University of California at San Francisco, and Stanley Cohen, at Stanford University, who used E. coli restriction enzymes to insert foreign DNA into plasmids. [12]

Recombinant DNA is the general name for a piece of DNA that has been created by the combination of at least two strands. Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure, and differ only in the nucleotide sequence within that identical overall structure. Recombinant DNA molecules are sometimes called **chimeric DNA**, because they can be made of material from two different species, like the mythical chimera. R-DNA technology uses palindromic sequences and leads to the production of sticky and blunt ends.

The DNA sequences used in the construction of recombinant DNA molecules can originate from any species. For example, plant DNA may be joined to bacterial DNA, or human DNA may be joined with fungal DNA. In addition, DNA sequences that do not occur anywhere in nature may be created by the chemical synthesis of DNA, and incorporated into recombinant molecules. Using recombinant DNA technology and synthetic DNA, literally any DNA sequence may be created and introduced into any of a very wide range of living organisms.

Proteins that can result from the expression of recombinant DNA within living cells are termed *recombinant proteins*. When recombinant DNA encoding a protein is introduced into a host organism,

the recombinant protein is not necessarily produced.[13]Expression of foreign proteins requires the use of specialized expression vectors and often necessitates significant restructuring by foreign coding sequences.[14]

Recombinant DNA differs from genetic recombination in that the former results from artificial methods in the test tube, while the latter is a normal biological process that results in the remixing of existing DNA sequences in essentially all organisms.

The Basics of Recombinant DNA[15]

That's a very good question! rDNA stands for recombinant DNA. Before we get to the "r" part, we need to understand DNA. Those of you with a background in biology probably know about DNA, but a lot of ChemE's haven't seen DNA since high school biology. DNA is the keeper of the all the information needed to recreate an organism. All DNA is made up of a base consisting of sugar, phosphate and one nitrogen base. There are four nitrogen bases, adenine (A), thymine (T), guanine (G), and cytosine (C). The nitrogenbases are found in pairs, with A & T and G & C paired together. The sequence of the nitrogen bases can be arranged inan infinite ways, and their structure is known as the famous "double helix" which is shown in the image below. The sugar used inDNA is deoxyribose. The four nitrogen bases are the same for all organisms. Thesequence and number of bases is what creates diversity. DNA does notactually make the organism, it only makes proteins. The DNA is transcribed into mRNA and mRNA is translated into protein, and the protein then forms theorganism. By changing the DNA sequence, the way in which the protein isformed changes. This leads to either a different protein, or an inactive protein.

Now that we know what DNA is, this is where the recombinant comes in.Recombinant DNA is the general name for taking a piece of one DNA, and combining it with another strand of DNA. Thus, the name recombinant! Recombinant DNA is also sometimes referred to as "chimera." By combining two or more different strands of DNA, scientists are able to create a new strand of DNA.The most common recombinant process involves combining the DNA of two different organisms.

How is Recombinant DNA made?

There are three different methods by which Recombinant DNA is made. They areTransformation, Phage Introduction, and Non-Bacterial Transformation. Eachare described separately below.

Transformation

The first step in transformation is to select a piece of DNA to be inserted into a vector. The second step is to cut that piece of DNA with a restriction enzyme and then ligate the DNA insert into the vector with DNA Ligase. The insert contains a selectable marker which allows for identification of recombinant molecules. An antibiotic marker is often used so a host cell without a vector dies when exposed to a certain antibiotic, and the host with the vector will live because it is resistant.

The vector is inserted into a host cell, in a process called transformation. One example of a possible host cell is E. Coli. The host cells must be specially prepared to take up the foreign DNA.Selectable markers can be for antibiotic resistance, colorchanges, or any other characteristic which can distinguish transformed hosts from untransformedhostsDifferent vectors have different properties to make them suitable to different applications. Some properties can include symmetrical cloning sites, size, and high copy number.

Non-BacterialTransformation

This is a process very similar to Transformation, which was described above. The only difference between the two is non-bacterial does not use bacteria such as E. Coli for the host.In microinjection, the DNA is injected directly into the nucleus of the cell being transformed. In biolistics, the host cells are bombarded with high velocity microprojectiles, such as particles of gold or tungsten that have been coated with DNA.

Phage Introduction

Phage introduction is the process of transfection, which is equivalent to transformation, except a phage is used instead of bacteria. In vitro packaging of a vector is used. This uses lambda or MI3 phages to produce phage

plaques which contain recombinants. The recombinants that are created can be identified by differences in the recombinants and non-recombinants using various selection methods.

Working of rDNA

Recombinant DNA works when the host cell expresses protein from the recombinant genes. A significant amount of recombinant protein will not be produced by the host unlessexpression factors are added. Protein expression depends upon the gene being surrounded by a collection of signals which provide instructions for the transcription and translationof the gene by the cell. These signals include the promoter, the ribosome bindingsite, and the terminator. Expression vectors, in which the foreign DNA is inserted,contain these signals. Signals are species specific. In the case of E. Coli, thesesignals must be E. Coli signals as E.Coli is unlikely to understand the signals ofhuman promoters and terminators. Problems are encountered if the gene contains introns or contains signals which act as terminators to a bacterial host. This results in premature termination, and the recombinant protein may not be processed correctly, be folded correctly, or may even be degraded. Production of recombinant proteins in eukaryotic systems generally takes place in yeast and filamentous fungi. The use of animal cells is difficult due to the fact that many need a solid support surface, unlike bacteria, and have complex growth needs. However, some proteins are too complex to be produced in bacterium,so eukaryoticcells must be used.

Importance rDNA

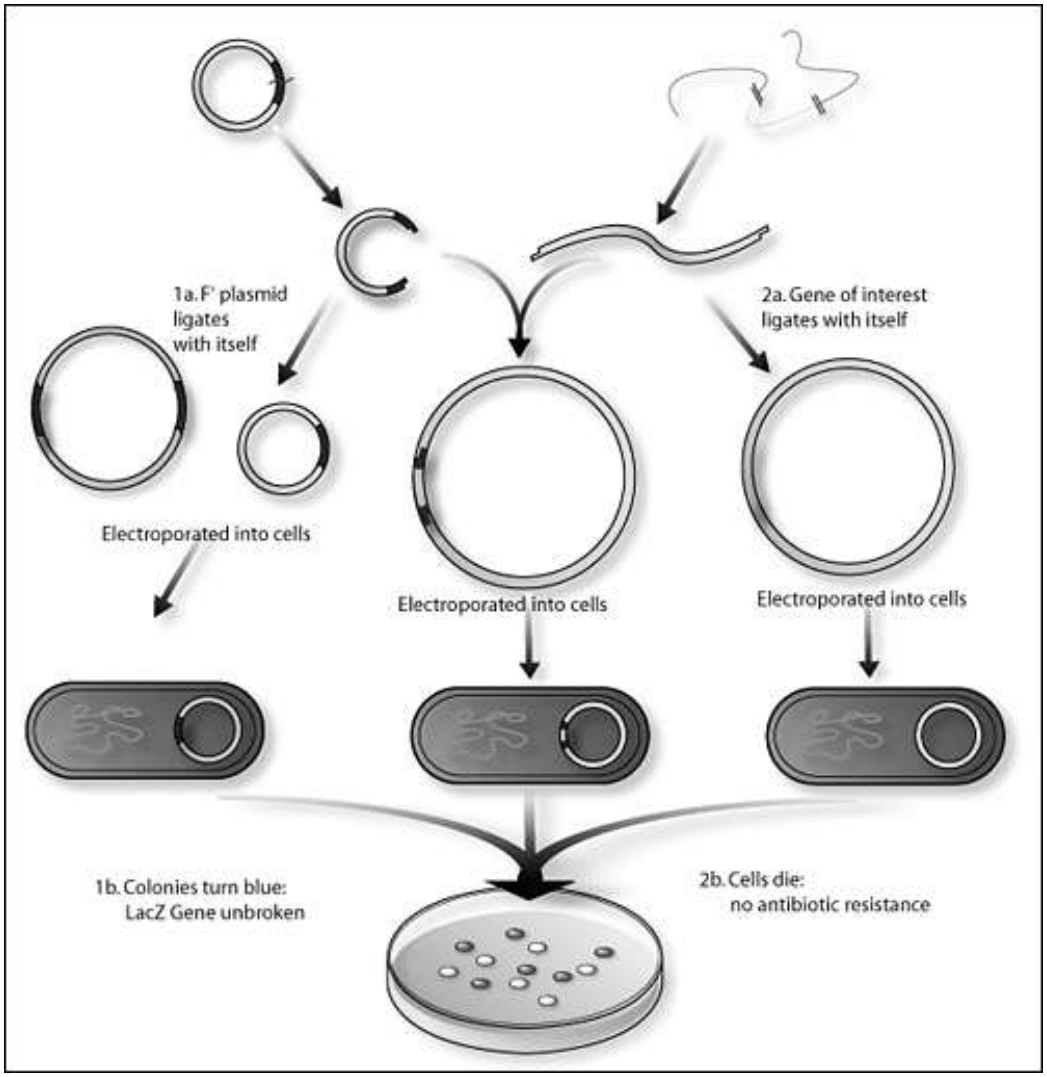
Recombinant DNA has been gaining in importance over the last few years, and recombinant DNA will only become more important in the 21st century as geneticdiseases become more prevelant and agricultural area is reduced. Below aresome of the areas where Recombinant DNA will have an impact.

- Better Crops (drought & heat resistance)
- Recombinant Vaccines (Hepatitis B)
- Prevention and cure of sickle cell anemia
- Prevention and cure of cystic fibrosis
- Production of clotting factors
- Production of insulin
- Production of recombinant pharmaceuticals
- Plants that produce their own insecticides
- Germ line and somatic gene therapy [15]
-

Future Prospectives

Now that we've figured out the basics behind what Recombinant DNA are, it'stime to look at how Recombinant DNA will impact the future. Which industries and fields will be shaped by rDNA? How will rDNA effect the health and lifestyles of RPI students in the next generation? Click over to our rDNA Impact Statement to find out the answer! Recombinant DNA Technology[16]

Recombinant DNA technology is a technique which changes the phenotype of an organism (host) when a genetically altered vector is introduced and integrated into the genome of the organism. So, basically, the process involves the introduction of a foreign piece of DNA structure into the genome which contains our gene of interest. This gene which is introduced is the recombinant gene and the technique is called the recombinant DNA technology. Inserting the desired gene into the genome of the host is not as easy as it sounds. It involves the selection of the desired gene for administration into the host followed by a selection of the perfect vector with which the gene has to be integrated and recombinant DNA formed. This recombinant DNA then has to be introduced into the host. And at last, it has to be maintained in the host and carried forward to the offsprings.



Recombinant DNA Technology[16]

Tools of Recombinant DNA technology

The tools mainly include the following:

1. **The enzymes which include the restriction enzymes** – help to cut, the polymerases- help to synthesize and the ligases- help to bind. The restriction enzymes used in recombinant DNA technology play a major role in determining the location at which the desired gene is inserted into the vector genome. They are two types, namely Endonucleases and Exonucleases. The Endonucleases cut within the DNA strand whereas the Exonucleases remove the nucleotides from the ends of the strands. The restriction endonucleases are sequence-specific which are usually palindrome sequences and cut the DNA at specific points. They scrutinize the length of DNA and make the cut at the specific site called the restriction site. This gives rise to sticky ends in the sequence. The desired genes and the vectors are cut by the same restriction enzymes to obtain the complementary sticky notes, thus making the work of the ligases easy to bind the desired gene to the vector.

2. **The vectors** – help in carrying and integrating the desired gene. These form a very important part of the tools of recombinant DNA technology as they are the ultimate vehicles that carry forward the desired gene into the host organism. Plasmids and bacteriophages are the most common vectors in recombinant DNA technology that are used as they have very high copy number. The vectors are made up of an origin of replication- This is a sequence of nucleotide from where the replication starts, a selectable marker – constitute genes which show resistance to certain antibiotics like ampicillin; and cloning sites – the sites recognized by the restriction enzymes where desired DNAs are inserted.

3. **Host organism** – into which the recombinant DNA is introduced. The host is the ultimate tool of recombinant DNA technology which takes in the vector engineered with the desired DNA with the help of the enzymes.

There are a number of ways in which these recombinant DNAs are inserted into the host, namely – microinjection, biolistics or gene gun, alternate cooling and heating, use of calcium ions, etc.

Steps in Recombinant DNA Technology[17]:

i. Selection and isolation of DNA insert
ii. Selection of suitable cloning vector
iii. Introduction of DNA-insert into vector to form rec DNA molecule
iv. rec DNA molecule is introduced into a suitable host.
v. Selection of transformed host cells.
vi. Expression and multiplication of DNA-insert in the host.

(i) Selection and isolation of DNA insert:

First step in rec DNA technology is the selection of a DNA segment of interest which is to be cloned. This desired DNA segment is then isolated enzymatically. This DNA segment of interest is termed as DNA insert or foreign DNA or target DNA or cloned DNA.

(ii) Selection of suitable cloning vector:

A cloning vector is a self-replicating DNA molecule, into which the DNA insert is to be integrated. A suitable cloning vector is selected in the next step of rec DNA technology. Most commonly used vectors are plasmids and bacteriophages.

(iii) Introduction of DNA-insert into vector to form recDNA molecule:

The target DNA or the DNA insert which has been extracted and cleaved enzymatically by the selective restriction endonuclease enzymes [in step (i)] are now ligated (joined) by the enzyme ligase to vector DNA to form a rec DNA molecule which is often called as cloning-vector-insert DNA construct.

(iv) rec DNA molecule is introduced into a suitable host:

Suitable host cells are selected and the rec DNA molecule so formed [in step (iii)] is introduced into these host cells. This process of entry of rec DNA into the host cell is called transformation. Usually selected hosts are bacterial cells like E. coli, however yeast, fungi may also be utilized.

(v) Selection of transformed host cells:

Transformed cells (or recombinant cells) are those host cells which have taken up the recDNA molecule. In this step the transformed cells are separated from the non-transformed cells by using various methods making use of marker genes.

(vi) Expression and Multiplication of DNA insert in the host:

Finally, it is to be ensured that the foreign DNA inserted into the vector DNA is expressing the desired character in the host cells. Also, the transformed host cells are multiplied to obtain sufficient number of copies. If needed, such genes may also be transferred and expressed into another organism.

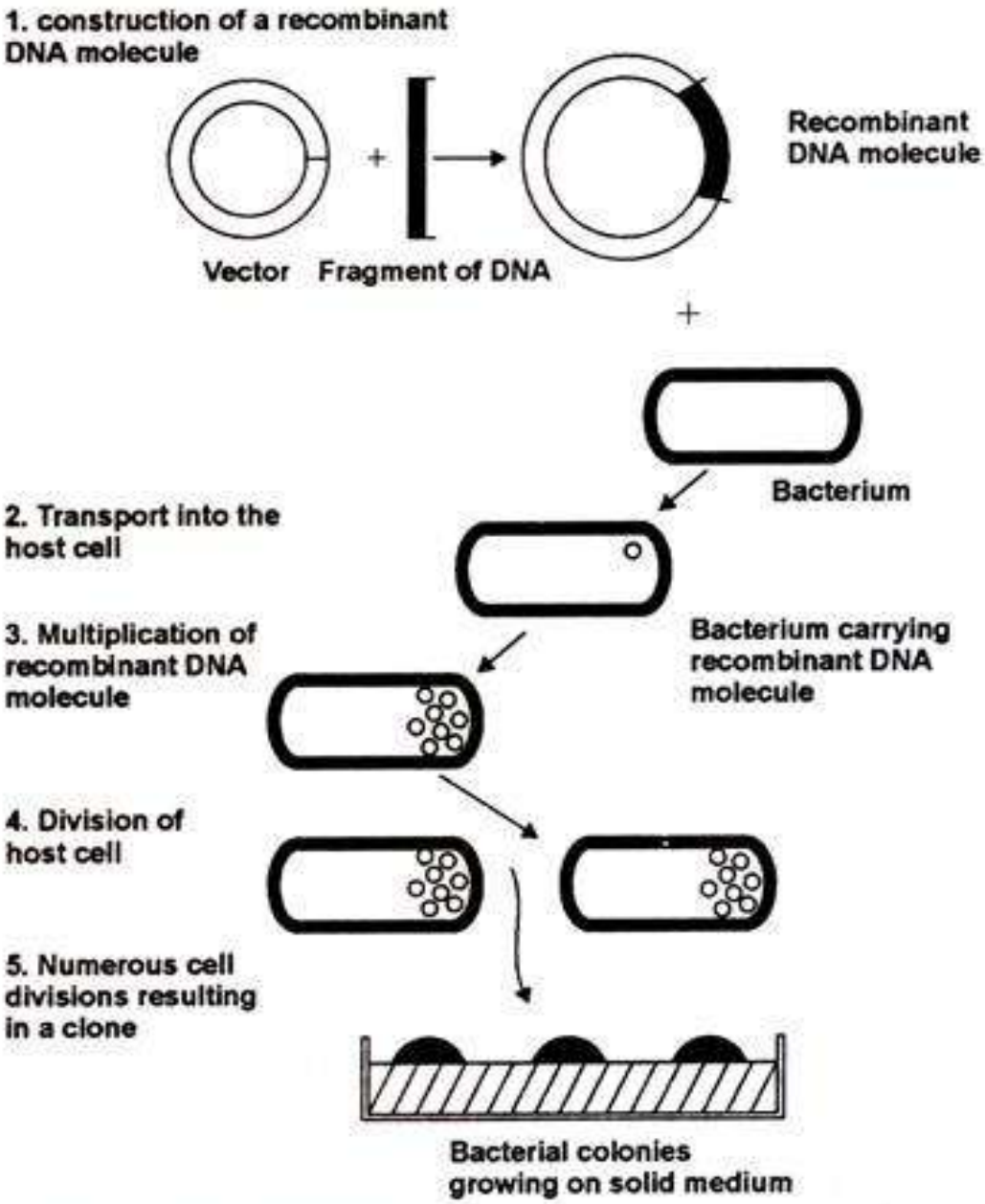


Fig. 1. The basic steps of rec DNA Technology using the bacterial plasmid as cloning vector.

Application of recombinant DNA technology[17]

1. Production of Transgenic Plants:By utilizing the tools and techniques of genetic engineering it is possible to produce transgenic plants or the genetically modified plants. Many transgenic plants have been developed with better qualities like resistance to herbicides, insects or viruses or with expression of male sterility etc.

2. Production of Transgenic Animals:By the use of rec DNA technology, desired genes can be inserted into the animal so as to produce the transgenic animal. The method of rec DNA technology aids the animal breeders to increase the speed and range of selective breeding in case of animals. It helps for the production of better farm animals so as to ensure more commercial benefits.

   Another commercially important use of transgenic animals is the production of certain proteins and pharmaceutical compounds. Transgenic animals also contribute for studying the gene functions in different animal species. Biotechnologists have successfully produced transgenic pigs, sheep, rats and cattle.

3. Production of Hormones:By the advent of techniques of rec DNA technology, bacterial cells like E.coli are utilized for the production of different fine chemicals like insulin, somatostatin, somatotropin and p-endorphin. Human Insulin Hormone i.e., Humulin is the first therapeutic product which was produced by the application of rec DNA technology.

4. Production of Vaccines:Vaccines are the chemical preparations containing a pathogen in attenuated (or weakened) or inactive state that may be given to human beings or animals to confer immunity to infection. A number of vaccines have been synthesized biologically through rec DNA technology, these vaccines are effective against numerous serious diseases caused by bacteria, viruses or protozoa. These include vaccines for polio, malaria, cholera, hepatitis, rabies, smallpox, etc. The generation of DNA vaccines has revolutionized the approach of treatment of infectious diseases. DNA-vaccine is the preparation that contains a gene encoding an immunogenic protein from the concerned pathogen.

5. Biosynthesis of Interferon:Interferon's are the glycoproteins which are produced in very minute amounts by the virus-infected cells. Interferon's have antiviral and even anti-cancerous properties. By recDNA technology method, the gene of human fibroblasts (which produce interferon's in human beings) is inserted into the bacterial plasmid.These genetically engineered bacteria are cloned and cultured so that the gene is expressed and the interferon's are produced in fairly high quantities. This interferon, so produced, is then extracted and purified.

6. Production of Antibiotics:Antibiotics produced by microorganisms are very effective against different viral, bacterial or protozoan diseases. Some important antibiotics are tetracyclin, penicillin, streptomycin, novobiocin, bacitracin, etc.recDNA technology helps in increasing the production of antibiotics by improving the microbial strains through modification of genetic characteristics.

7. Production of Commercially Important Chemicals:Various commercially important chemicals can be produced more efficiently by utilizing the methods of rec DNA technology. A few of them are the alcohols and alcoholic beverages obtained through fermentation; organic acids like citric acid, acetic acid, etc. and vitamins produced by microorganisms.

8. Application in Enzyme Engineering:As we know that the enzymes are encoded by genes, so if there are changes in a gene then definitely the enzyme structure also changes. Enzyme engineering utilizes the same fact and can be explained as the modification of an enzyme structure by inducing alterations in the genes which encode for that particular enzyme.

9. Prevention and Diagnosis of Diseases:Genetic engineering methods and techniques have greatly solved the problem of conventional methods for diagnosis of diseases. It also provides methods for the. prevention of a number of diseases like AIDS, cholera, etc. Monoclonal antibodies are useful tools for disease diagnosis. Monoclonal antibodies are produced by using the technique called hybridoma technology.

10. Gene Therapy:Gene therapy is undoubtedly the most beneficial area of genetic engineering for human beings. It involves delivery of specific genes into human body to correct the diseases. Thus, it is the treatment of diseases by transfer and expression of a gene into the patients' cells so as to ensure the restoration of a normal cellular activity.

11. Practical Applications of Genetic Engineering:recDNA technology has an immense scope in Research and Experimental studies.

    It is applied for:

    a. Localizing specific genes.

    b. Sequencing of DNA or genes.

    c. Study of mechanism of gene regulation.

    d. Molecular analysis of various diseases.

    e. Study of" mutations in DNA, etc.

12. **Applications in forensic science:** The applications of rec DNA technology (or genetic engineering) in forensic sciences largely depend on the technique called DNA profiling or DNA fingerprinting. It enables us to identify any person by analysing his hair roots Wood stains, serum, etc. DNA fingerprinting also helps to solve the problems of parentage and to identify the criminals.

13. Biofuel Production: Biofuels are derived from biomass and these are renewable and cost effective. Genetic engineering plays an essentially important role in a beneficial and largescale production of biofuels like biogas. bio hydrogen biodiesel bio-ethanol., etc. Genetic engineering helps to improve organisms for obtaining higher product yields and product tolerance.

14. Genetically stable high producing microorganisms are being developed by using modern recDNA techniques, which aid in an efficient production of bioenergy.

15. The energy crop plants are those plants which use solar energy in a better way for production of biomass. Genetic improvements of these energy crop plants greatly help for quick and high Product on of biomass which in turn reduces the biofuel production cost. The fermenting microbes which are utilized for biogas production are improved at the genetic level for achieving better result.

16. Environment Protection:Genetic engineering makes its contributions to the environment protection in various ways. Most important to mention are the new approaches utilized for waste treatments and bioremediation Environment protection means the conservation of resources and hence to limit the degradation of environment.

References

1. Kumar S., Kumar A. Role of genetic engineering in agriculture. *Plant Archives*. 2015;15:1–6.
2. Cardi T., Stewart C. N., Jr. Progress of targeted genome modification approaches in higher plants. *Plant Cell Reports*. 2016;35(7):1401–1416. doi: 10.1007/s00299-016-1975-1.
3. Lomedico P. T. Use of recombinant DNA technology to program eukaryotic cells to synthesize rat proinsulin: a rapid expression assay for cloned genes. *Proceedings of the National Academy of Sciences of the United States of America*. 1982;79(19):5798–5802. doi: 10.1073/pnas.79.19.5798.
4. Ullah M. W., Khattak W. A., Ul-Islam M., Khan S., Park J. K. Encapsulated yeast cell-free system: a strategy for cost-effective and sustainable production of bio-ethanol in consecutive batches. *Biotechnology and Bioprocess Engineering*. 2015;20(3):561–575. doi: 10.1007/s12257-014-0855-1.
5. Ullah M. W., Khattak W. A., Ul-Islam M., Khan S., Park J. K. Bio-ethanol production through simultaneous saccharification and fermentation using an encapsulated reconstituted cell-free enzyme system. *Biochemical Engineering Journal*. 2014;91:110–119. doi: 10.1016/j.bej.2014.08.006.

6. Khattak W. A., Ul-Islam M., Ullah M. W., Yu B., Khan S., Park J. K. Yeast cell-free enzyme system for bio-ethanol production at elevated temperatures. *Process Biochemistry*. 2014;49(3):357–364. doi: 10.1016/j.procbio.2013.12.019.
7. Khattak W. A., Ullah M. W., Ul-Islam M., et al. Developmental strategies and regulation of cell-free enzyme system for ethanol production: a molecular prospective. *Applied Microbiology and Biotechnology*. 2014;98(23):9561–9578. doi: 10.1007/s00253-014-6154-0.
8. Galambos L., Sturchio J. L. Pharmaceutical firms and the transition to biotechnology: a study in strategic innovation. *Business History Review*. 1998;72(2):250–278. doi: 10.2307/3116278.
9. Steinberg F. M., Raso J. Biotech pharmaceuticals and biotherapy: an overview. *Journal of Pharmacy and Pharmaceutical Science*. 1998;1(2):48–59.
10. Liu W., Yuan J. S., Stewart C. N., Jr. Advanced genetic tools for plant biotechnology. *Nature Reviews Genetics*. 2013;14(11):781–793. doi: 10.1038/nrg3583.
11. Venter M. Synthetic promoters: genetic control through cis engineering. *Trends in Plant Science*. 2007;12(3):118–124. doi: 10.1016/j.tplants.2007.01.002.
12. "GNN- Genetics and Genomics Timeline". www.genomenewsnetwork.org. Retrieved 16 February 2018.
13. *Rosano, Germán L.; Ceccarelli, Eduardo A. (2014-04-17).* "Recombinant protein expression in Escherichia coli: advances and challenges". *Frontiers in Microbiology. 5*. doi:10.3389/fmicb. 2014.00172. ISSN 1664-302X. PMC 4029002. PMID 24860555.
14. "Promoters used to regulate gene expression". *www.cambia.org*. Retrieved 16 February2018.
15. www.rpi.edu/dept/chem-eng/Biotech-Environ/Projects00/rdna/rdna.html
16. byjus.com/biology/recombinant-dna-technology/
17. http://www.biologydiscussion.com/dna/recombinant-dna-technology/recombinant-dna-technology-with-diagram/17785

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