

Technology of recombinant dna



The technology of recombinant dna is a DNA based major tool that has acquired popularity and lots of attention in the present world. With the use of this technology, scientists are able to get individual genes from desired sources of either plant or animal nature, cleave or cut them out and finally use them in the target organism by inserting the isolated gene into its genome to transfer the desired characteristics. To be able to carry out recombinant DNA, it is very vital for one to have restriction enzymes which are commonly known as molecular scissors. These restriction enzymes are used to cleave the DNA genes by cutting them at specific predetermined sequence. After cutting the genes, the cut genes are carefully transferred and inserted into a plasmid which is a piece of circular bacterial DNA. This is then followed by re-introduction of the plasmid to a bacterial cell. The logic is that the introduced plasmid cell will start to multiply as long as the bacterial cell multiplies and this will eventually give rise to lots of gene copies. With the fact that most bacterial cells multiply very rapidly, this idea can be used or applied in the modern laboratories to generate or bulk up useful genes to suit many application needs and also for further analysis. Basically recombinant DNA is composed of deoxyribonucleotides molecules which are segments of DNA obtained from at least two sources. The science recombining DNA has always been in existence happening only in a natural phenomenon until recently when researchers discovered and came up with a way to carry out this DNA recombination in the laboratory. Generally, DNA is defined as a heredity molecule and the method employed in preparation of recombinant DNA is termed as genetic engineering. The discovery of recombinant DNA has been a blessing to many fields and industries as it has find its way in field of agriculture, medicine, biotechnology and basic

research. The technology has been applied in medicine to come up with pharmaceutical products like human insulin. In the field of agriculture, the technology has been used to transfers specific desirable characteristics from one plant to another to improve on the resistance to diseases, tolerance to drought, yield increase and sometimes to impart rich nutritional content (Garrett, Reginald, and Grisham 254)

How the technology of recombinant DNA developed

The development of the technology of recombinant DNA was enabled by discovery of cleavage enzymes whose purpose is to cut at specific sequences the double stranded DNA molecules and these enzymes are known as restriction endonucleases. These restriction endonucleases are enzymes normally produced by bacteria purposely to protect it from attack or invasion by bacterial viruses and the production of these enzymes is induced by the bacterium's defense mechanism and they act by means of destruction of the viral DNA. The process of cutting or cleavage of the DNA strands at specific sites is termed as hydrolysis and this result into single strands of DNA molecules and this strand have ends which are sticky and this is because of the cut's asymmetry that was done to the DNA molecule when still double stranded. The stickiness is also brought about due to the tendency of formation of hydrogen bonds by the DNA bases. The bases of one DNA strand tend to bond with the complementary bases of another DNA strand to form these hydrogen bonds. During the research, the scientists discovered that these particular enzymes can be give good results when applied as a tool to manipulate DNA in a controlled process. The concern by many scientists and other researchers to whether this new technology of

recombinant DNA has risks related to the environment, human beings or not led to a temporary and unprecedented suspension of all experiments that employed the use of recombinant DNA. A conference was then later held in California, Asilomar in 1975 to carry out assessment to such risks. The outcome of this conference was that a decision was made that most of the work involving the use recombinant DNA should continue to be carried out so long all the safety measures and precautions that should be taken when carrying out the process are well and strictly adhered to. An advisory committee on recombinant DNA was then set up and its main mandate was to come up with guidelines and also engage in assessment of the possible benefits and the risks associated with the recombinant DNA projects proposed. This committee was enacted by the national health institute of California. The selected committee was made up scientist researchers, legal experts and physicians. The committee also included ethicists. The committee has had several meetings since then (Fredrickson 271).

Recombinant DNA Therapy.

The technology of recombinant DNA has brought about revolution in the field of biology and its current impact to clinical medicine is on the rise. Pedigree analysis has elaborated much on the genetic human diseases and the associated proteins affected but still the technology of recombinant DNA can assist a lot especially in situations where there is unknown specific genetic defect since the technology is able to overcome such limitations by obtaining information directly from the DNA molecule. The whole idea revolves around manipulation of the sequence of DNA molecules and chimeric molecules

formation and this will help in better understanding of the working of DNA specific segments of the molecules.

Recombinant DNA technology is a form of genetic engineering which is involved in manipulation of the genetic make up of the selected organism by introducing foreign DNA genes isolated from another organism and this is achieved by experimental techniques. The tools employed during genetic engineering process are as follows. The first one is enzymes like the restriction enzymes which act as scissors to cleave or cut the DNA molecules at specific site as desired for various applications. The second one is passenger DNA. A passenger DNA is a foreign DNA that is transferred from the source organism to the target organism in a passive form. Examples of passenger DNA include synthetic DNA, complementary DNA and many others. The third and last tool used in genetic engineering process is called the vehicle or the vector DNA. This is the kind of DNA that serves a role as the transfer medium of the gene from the source to the target organism. It acts as a carrier as a vehicle for this application. Examples of vehicle or vector DNA include bacterial phages, cosmids, and bacterial plasmid among others (Eun, Hyone and Myong 178).

Stages of recombinant DNA Application Technology.

Specific DNA isolation. Since the size of the genomic DNA is large, the specific required fragments of this genome can be isolated by means of cleavage or splicing and this process is enabled by a group of enzymes known as restriction endonucleases. Restriction endonucleases can be best described to be chemical knife which cut or cleave the DNA molecule at

specific sites to form require DNA sequences and these enzymes are the most vital in genetic engineering.

Hybrid or chimeric DNA. Since the main purpose of carrying out recombinant DNA is to insert the passenger DNA of interest into the vector or vehicle DNA to enable replication of the DNA of interest along with the vehicle DNA after the annealing process. It is this process of hybrid combining of two segments of DNA that is termed as recombinant DNA or chimeric DNA.

Steps for hybrid/chimeric DNA preparation

The required plasmid is first cut in circular form by means of endonucleases which are restriction enzymes. In case of use of Eco R 1 restriction enzyme, the sticky ends of the two DNA strands will have a genetic coding of AATT AND TTAA respectively.

The insert DNA or the passenger DNA is also cut by the endonucleases used in step one and this is mainly to obtain uniformity in the sequence obtained from the sticky ends of the cleaved piece.

This is followed by incubation of the cut piece of human DNA together with the vector DNA and this is mainly to allow annealing to take effect. Since the sticky ends of both the human DNA and the vector DNA have complementary sequences, they will attract and stick together.

Then the ligase DNA enzyme is allowed to take action on the chimeric or hybrid DNA. This enzyme acts by creating a link between the insert and the vector molecules by covalent phosphodiester bonds.

Cloning of chimeric DNA. A clone is described as large population of bacteria, cells or molecules that are identical arising from a single common ancestor. The main objective of DNA cloning is to generate large numbers of identical molecules of DNA for various applications (Watson D. J Et al. 334).

Application of Recombinant DNA in Medicine.

Recombinant DNA has led to development of various medical products.

Human growth hormone and insulin were the first two products of recombinant DNA technology prepared commercially. The E. coli bacterium was used to culture both of these products. Since the development of these two products, there has been a lot of other products appearing in markets made using recombinant DNA technology. Examples of products made in E. coli include Tumor Necrosis Factor which has application in treatment of tumor cells. The second product is called Interleukin-2 and this one has effect in treatment of cancer and deficiency of immunity. It is also used as an anti-retroviral therapy in treatment of HIV infection. The third one is Prourokinase which is very effective in treatment of heart attach and other heart related diseases. The fourth one is called Taxol and this one is used in treatment of ovarian cancer. The last one is termed as Interferon and this recombinant DNA product is used to correct cancer and other forms of viral infection.

A vaccine can be described as a bacterium version or a virus introduced into an organism and is meant to activate the immune system of that organism against attack and in future, it is capable of destruction of such similar substances. Several vaccines are now days prepared commercially using

recombinant hosts. Previously, the mode of preparation of a vaccine was by first denaturing the disease followed by injection into the body of human being with an assumption that it will help boost the immunity system of the individual and be able to fight such similar intrusions in future. But this did not work as desired since the patient tested positive for that particular disease after some time. But with the introduction of modern technology of recombinant DNA, only the outside identifiable outer covering or shell of the microbe required, copied and finally injection is done to a host that is harmless to come up with a vaccine. This method is considered to be likely a much or more safer technology and this is mainly because it does not involve the transfer of the actual microbe that is causing the disease to the host. Since the system of immunity is normally activated by the specific types of protein only found on the surface of the microbe, this technology of DNA takes into consideration this fact and therefore makes use of only identifiable microbe surface features for preparation of the vaccine. Speaking of this, at the moment, the types of vaccines that are being worked on to be tried for effectiveness are those of malaria, hepatitis B virus and type 2 herpes and these ones are expected to take effect in the near future.

In the modern medicine sector, recombinant DNA is much linked. It has found application in gene therapy which involves replacement of defective genes with those that are still functional and these are introduced into a patient by a vector that is appropriate and in most cases is a virus that is disabled. The first gene therapy that was moderately successful was applied in treatment of ADA deficiency which is also termed as inborn immunity deficiency disorder. Recombinant DNA has also been applied in development

of vaccines and cancer treatments as discussed before. Recently, the researchers are working toward making it possible that organs appropriate for transplant from genetically modified animals to humans are harvested. This is still in progress (Russell David Et al. 415).

Application of Recombinant DNA in Agriculture

Over the past couple of years, the focus of biotechnology has been and still is on the plant crops. And tea in areas of focus improvement is in terms of the yield or out put produced and the capability of the crop to encounter and be able to resist certain destructive diseases. The other areas of concern especially for fresh fruits and vegetables are delayed ripening to better the transport capability of the products with few cases of injuries and also spoilage resistance. The process of coming up of plants that had artificially obtained genes from another crop also called transgenic plants has long proved to be a much difficult and complicated process as compared in the case of animals. It was until the discovery of isolation of the Ti plasmid which is found in the tumor inducing bacteria that mostly exists in the soil that a vector was able to be found for plant genetic engineering otherwise the whole process could not go anywhere without this vector. The isolation of this vector has also proved to be a hectic process. The plasmid is introduced into a cell and once introduced, the plasmid then attaches to the plant cell's DNA readily. The Ti plasmid has shown very minimal success in grain crops although the results with fresh fruits and vegetables have been successful.

The application of recombinant DNA to a sector of agricultural herbicides also proved to be a success as the researchers were able to come up with a plant that is resistance to a specific herbicide and eventually the use of the

herbicide got rid of the weed reducing weed competition with the desired crop plant. These researchers were able to identify the bacteria that are resistant to the herbicide then carried out the isolation of the genes responsible for this state or condition and finally introduced them into a plant using the appropriate vector. Eventually the process proved to be effective since the crop plant showed resistance to the herbicide used (Singer and Soll 1114). This technology has improved to a level where researchers are coming up with similar plant crops but this time resistance to insects and this is mainly due to discovery of bacterial enzymes that are detrimental to herbivores that are unwanted or immobilize them. Others also have been found to fix nitrogen into the soil for plant use.

Considering the idea of nitrogen fixation, most of the plants need nitrogen as basic nutrient for proper growth. Although the atmosphere is full of nitrogen to almost 78%, the form in which this nitrogen exists can not be utilized by plant. The rhizobium bacteria which is found to occur naturally in the soil and in some leguminous plants has been found to be able to convert the atmospheric nitrogen into a form that plants can utilize. Basing on this discovery, the researchers are hoping and working towards isolation of the specific gene from these rhizobium bacteria and identifying the specific segment of the DNA that is responsible fixation of nitrogen. After this, they can remove it and introduce it into the DNA of the targeted crop or even a cash crop which is much profitable. With doing this, the created transgenic crop will be able to thrive well in nitrogen deficient soils and many other territories not suitable for their growth. The cost of fertilizers will also be cut down (Mertz and Davis 3372).

Conclusion

With further research and fully implementation of recombinant DNA findings especially in field of medicine and agriculture, very many problems like food insecurity and inadequacies in vaccines and other medical requirements can be solved or minimized provided the safety precautions are well observed to avoid the risks and hazards related.

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