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(Author)

Use of Restriction Enzymes in Pharmaceuticals
Restriction enzyme, which is also referred to as restriction endonuclease, is a class of enzyme that is helpful in cutting DNA at restriction sites. Restriction sites are particular recognition nucleotide sequences on double stranded DNA having the length of 4-5 base pairs. Restriction enzymes are also known deoxyribonucleases (DNases). Presently, there are four different types of restriction enzymes:
- Type I enzymes that are “ classical” in nature, e. g. EcoKI, EcoBI, and EcoR124;
- Type II enzymes that are “ orthodox” in nature, e. g. EcoRI, HindIII, EcoRV;
- Type III enzymes having more than one subunits, e. g., EcoP1I and EcoP15I; and
- Type IV such as modification-dependent restriction enzymes, McrBC and Mrr systems
Type V restriction enzymes e. g., cas9-gRNA complex from CRISPRs have also been introduced recently. Artificial restriction enzymes, e. g. Zinc-finger nucleases (ZFN) and the TAL-effector nucleases have also been designed by fusing a natural or engineered DNA binding domain to a nuclease domain. They are of help in gene targeting and gene therapy (Loenen 9).
These restriction enzymes are different from each other as some of them are able to have the same recognition and cleavage sites, and others have different recognition and cleavage sites. These enzymes are commonly found in bacteria and archaea, and help them in protection from viruses. By the year 2007, more than 3, 000 restriction enzymes were studied by scientists in detail, and over 600 of those enzymes were available commercially (Roberts D269–D270). In 2013, more than 19, 000 putative restriction enzymes were listed on the restriction enzyme database known as REBASE. More than 300 Type II restriction enzymes are commercially available (Loenen 1).
Usually, pharmaceutical and biotechnological companies work on restriction enzymes as they are quite expensive for an individual scientist. They are placed in a small tube within freezer as it is important to keep them refrigerated all the time. These enzymes are handled very carefully, after wearing gloves, as the solution containing these enzymes could be contaminated by the addition of other DNA or enzymes that can alter, reduce or remove the activity of these enzymes.

## Applications of Restriction enzymes

Without the existence of restriction enzymes, many processes of modern technology such as biotechnology and genetic engineering would not be possible. Restriction enzymes are used either as primary sources or secondary sources in the processing and/or verification of various technologies such as recombinant DNA technology, polymerase chain reaction (PCR) based cloning and DNA fingerprinting. PCR is the process in which thousands to millions of copies of a specific DNA sequence are produced. DNA fingerprinting is a technique that is used by scientists to help in isolation of sequences of DNA and identification of individuals by their respective DNA profiles. These technologies are also used by pharmaceutical industries in one way or the other.

## Use of Restriction enzyme in Medical Industry

Restriction enzymes are used as tools in the development of medical products – many of them are also important from pharmaceutical point of view. They have helped in getting a good amount of information about DNA–protein interactions, associations and catalysis, protein family relations, control of restriction activity and flexibility of protein domains, and giving essential tools for research in molecular biology and personalized medicine (Loenen 1). Many biological and biopharmaceutical processes are dependent on the interaction and association of proteins with multiple DNA sites and drugs (Szczelkun 404). Therefore, they can help in study of drugs in the body.
These enzymes can also be used to differentiate gene alleles by specific recognition of single base changes in DNA known as single nucleotide polymorphisms (SNPs). This is possible only when SNP changes the restriction site in the allele. Alleles with changed restriction sites cannot be cut. Restriction enzymes are also used to digest genomic DNA for gene analysis by the technique of Southern blot. This technique helps in determination of copies of a gene in the genome of an individual, or the number of genetic mutations that have occurred within a population.

## Uses of Restriction Enzymes from Pharmaceutical Point of View

Pharmaceutical companies are working on the advanced techniques of biotechnology and restriction enzymes in order to develop better chemicals and drugs. However, in pharmaceutical industry, recombinant DNA technology has contributed a lot and helps in transforming the field of pharmaceutical technology and medicine. It has helped in the production of a large number of pharmaceutically important products (examples are mentioned below in the paper).
Pharmaceutical trends are inclined towards two most important technologies. One is the development of Novel Drug Delivery systems and the other is the use of Genetic engineering especially recombinant genetic technology. Genetic engineering has made it possible to biologically produce therapeutically important proteins on large-scale. Pharmaceutical industry has successfully combined the conventional and novel medical technologies to improve the ability of microorganisms in producing conventional antibiotics and to obtain new antibiotics (Zachariah, and Leena, ispub. com).
Recombinant DNA technology in the production Of Pharmaceuticals. Recombinant DNA technology is particularly associated with the Type II restriction enzymes. So, Type II restriction enzymes are of particular interest for pharmaceutical Research and Development. Type II enzymes has given many practical advantages, as K12 strain of E. coli, its genes and its vectors, are helpful in the detection and overproduction of hormones and enzymes (Loenen 9). Some of the restriction enzymes such as EcoRI and HindIII are also found to have a special role in the development of recombinant DNA (Loenen 3).
HaeIII, a type of restriction enzyme obtained from the Haemophilus aegyptius bacteria, is used by pharmaceutical industries in the production of human growth hormones.
Restriction enzymes have also helped in the production of insulin from recombinant bacteria and yeast by Genentech and the manufacture of a recombinant vaccine for Hepatitis B by Biogen (Loenen 9). For a long time, insulin was taken by purification of insulin from the pancreas of cows and pigs. However, it was a time-consuming and expensive phenomenon. Now, restriction enzymes and their use in genetic engineering help scientists to make genetically-engineered microorganisms that can give large quantities of insulin that can be purified and used. In this process of genetic engineering, plasmid (a small piece of DNA) is obtained from bacterium. This plasmid is cleaved by restriction enzymes and human insulin gene is inserted into the plasmid. This plasmid is referred to as genetically-engineered plasmid that is then inserted into the new bacterium. This new bacterium divides and starts giving insulin for human use.
With the help of restriction enzymes; recombinant DNA technology and other biotechnological procedures have made it possible to produce a number of human proteins in vitro. Genetically-engineered cells such as E. coli, yeast, and mammalian cells have made it possible to develop over 100 pharmaceutically important products for human therapy. Some examples of these medical innovations are
- parathyroid hormones,
- hepatitis B surface antigen (HBsAg), which is used in vaccination against the hepatitis B virus,
- factor VIII for people having the problem of hemophilia A,
- factor IX for people with the problem of hemophilia B,
- erythropoietin (EPO) for the treatment of anemia,
- tissue plasminogen activator (TPA) for dissolution of blood clots, and
- many monoclonal antibodies.
Use of Gene Coding and Cloning in Pharmaceutical industry. Restriction enzymes have become one of the most important tools in pharmaceutical and biotechnology research. They are commonly used for modification in DNA in laboratories, and are of significant help in molecular cloning. These enzymes are helpful in insertion of genes into plasmid vectors during the process of gene cloning and protein expression. Gene coding for important metabolic proteins and enzymes can be cloned into antibiotics producing microbes resulting in improved biosynthetic pathways to enhance the production of antibiotics through genetic technologies. Research examples are as follows (Zachariah, and Leena, ispub. com).
- Successful utilization of the recombinant DNA technology to transfer acyltransferase genes among different species of bacteria to get solvent extractable cephalosporins; and
- The combination of different genes through recombinant DNA technology and transformation for efficient production of the antibiotic amikacin.
Use of the bacterial restriction modification system in Pharmaceutical industry. Scientists have also suggested the use of the bacterial restriction modification system as a model to design novel anti-viral genes or genomic vaccines and therapeutic strategies. The restriction modification system is used by bacteria and some other types of prokaryotes to protect themselves from foreign DNA as, for example, DNA of bacteriophage. Scientists are working on artificial restriction enzymes such as ZFN to cut the DNA of different human viruses such as HSV-2, high-risk HPVs and HIV-1, to induce mutagenesis and aberrations of human-infecting viruses.
Restriction enzymes in the detection of certain diseases. Restriction enzymes are helpful in monitoring Restriction Fragment Length Polymorphisms (RFLP) permitting the identification of disease causing genes such as Huntington disease and sickle cell problems. Restriction enzymes are also helpful in identification of pathogenic strains of bacteria such as S. aureus sp including the methicillin-resistant S. aureus (MRSA) bacteria having virulence factors and the ability to resist antibiotics (Loenen 10). These MRSA bacteria have unique Type I restriction modification systems (Roberts 7472), and are of great threat to humanity. Working on these systems could help pharmaceutical companies to develop better drugs against these pathogens.
Restriction enzymes can help in further pharmaceutical research. Researchers have done a lot of work on restriction enzymes as biotechnological tools. They are also working a lot, but basic study of their behavior in their natural hosts need further investigations. It has been suggested that study of the actions of translocating enzymes such as the Type I and IV enzymes could further help in the development of novel medicines. In this case, action of enzymes at different components or structures is a most important topic of interest in medicine (Loenen 3). Another idea for research is that they can be used in the development of better antiaging medicines as, for example, GIY-YIG endonucleases, which are successfully used in the maintenance and repair of DNA (Mak 1321).

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