Recent Advancement in Genetic engineering using DNA manipulating enzymes

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***Abstract*: The development of molecular genetics as well as genetic manipulation are leading to innovative changes in the field of life science. Genetic engineering is an accurate technique of gene manipulation at the molecular level. Several enzymes play an important role in genetic engineering like restriction endonuclease, ligase, Alkaline phosphatase as well as DNA polymerase. The importance of genetic engineering in medical science consists of the formation of vaccines, growth hormones including proteins along with the treatment of various diseases. Genetic engineering has several applications in agriculture, research, medicine as well as industrial biotechnology. CRISPR/Cas9 is the new genome editing technique with excellent advantages. The fast advancement in CRISPR/Cas9 system greatly revolved around genetic engineering, particularly modifications of the Cas9 as well as sgRNA to attain proper editing.**

***Index Terms*— Genetic Engineering, CRISPR-Cas9, DNA Manipulation, Genome**

INTRODUCTION

Genetic engineering provides a great advantage in the research area begins to be developed in 1973 by Stanley Cohen with Herbert Boyer [1]. Gene manipulation including genetic engineering is in used for a very long time. Genetic engineering (GE) also known as gene manipulation as well as recombinant DNA technology (RDT) three are used as a conversely-intimate of manipulation as well as interchange the genetic constituents of the organism by introducing some traits of the interest [2]. This is the combination of methods that are useful for the recognition, replication, modification as well as transfer of genetic material. The methods specified above are through including great in the area since the 1970s with various importance in areas such as pharmaceuticals, industry, health, the environment in addition to agriculture. With the development of genetic engineering, its importance field is expanded that provide benefit to humans several genetic disorders is nowadays curable with the help of this technique it is not available before time. The importance of genetic engineering in medical science consists of the formation of vaccines, growth hormones including proteins along with treatment of various diseases including genetic disorders like Cancer, Down's syndrome, Alzheimer's disease, cystic fibrosis including Huntington's disease with the help of gene therapy. The Transgenic plants the useful traits like resistance to pests, herbicide tolerance, stress tolerance as well as increased nutrients. In the agriculture field which importance of GE is main apparent in developing genetically modified (GM) foods [3]. Genetic engineering is also used to enhance milk as well as meat this is the "values added" initiative in the area of animal agriculture that is enhanced the number of existing proteins by developing completely new ones [4]. Genetic engineering is the main important technique for the study of genomes, the formation of genetically modified (GM) organisms is formed by proteins of biotechnological importance and advancements of the transgenic organisms by new characteristics [5]. CRISPR/Cas9 is the growing essential technique for genome engineering because of its great efficiency as well as simplicity [6]. The rapid advancement in CRISPR/Cas9 technology greatly revolved around genetic engineering, particularly modifications of the Cas9 as well as sgRNA to attain proper editing [7].

ENZYMES IN DNA MANIPULATION

Nucleic acids are widely manipulated, to obtain particular attributes as well as features. The modifications are mainly catalyzed with the enzymes

like polymerases, and ligases including nucleases. The DNA polymerase enzyme synthesizes DNA molecules from deoxyribonucleotides. The various types of DNA polymerase are useful for gene manipulation. DNA polymerase I involved 3'-5' as well as 5'-3' exonuclease both actions including 5'-3' polymerase action. Reverse transcriptase synthesizes DNA from RNA. Taq DNA polymerase is a heat-stable DNA polymerase and is mainly extracted with Thermus aquaticus. That work at 72°C with slightly stabilized over 90°C and it mainly functions in PCR. Nucleases are enzymes that degrade nucleic acids by breaking the phosphodiester bonds. Ribonucleases (generally abbreviated RNases) mainly catalyzed the degradation of RNA including deoxyribonucleases (generally abbreviated DNases) that mainly catalyzed the degradation of DNA. Nucleases consist of two different types such as exonucleases as well as endonucleases. Exonucleases that cleave off nucleotides one at a time from 3'or 5' ends of DNA as well as RNA chains. The endonucleases cleave the phosphodiester bonds within a nucleic acid. Mung bean nucleases are the endonuclease particular from single-stranded DNA including RNA. The S1 nuclease is the endonuclease that degrades ssDNA and RNA. RNase H is an enzyme that cleaves the RNA of an RNA-DNA duplex [8]. The restriction-modification system consists of a modification enzyme (DNA methylase) that identifies the recognition regions including modifying the regions with the addition of a methyl group. The bacteria produce restriction enzymes as well as modification enzymes that modify their DNA because of that it will not be cleaved with a restriction endonuclease. The reason for modification is enzyme modifies the recognition sites, due to that restriction enzymes are incapable to cut those sites. So, with the help of modification systems protect host cell DNA from restriction enzymes [9]. Hamilton discovered the first restriction enzyme Hind III in 1970 from the Haemophilus influenza. Restriction enzymes is identifying a particular sequence and cut the DNA strand. Enzymes are classified into various types that are different primarily on the cofactor demands, structure, activity, and recognition as well as cleavage sites. The three different types of restriction endonucleases like Type I, II including III. The Type II enzyme performs an important function in Recombinant DNA Technology which consists of the simple structure of the protein, and for the cleavage of DNA, ATP is not necessary [10]. Table [11]. End modification enzymes make changes to the ends of DNA molecules. Enzyme terminal deoxynucleotidyl transferase addicts the series nucleotides onto 3'OH termini of dsDNA molecule [12]. Alkaline phosphatase was found in E. coli as well as calf intestinal tissue, which catalyzed the removal of phosphate groups of 5'ends of the DNA strand [13]. T4 polynucleotide kinase, which is mainly isolated from the E. coli cells infected by the T4 phase, carries out a reverse reaction of the alkaline phosphatase, the addition of phosphates to the 5' ends [14].

ADVANCEMENTS IN GENETIC ENGINEERING OF CRISPR/CAS9 SYSTEMS

The Genetic engineering technique is greatly involved in the area of life sciences research as well as numerous importance. In 1987, CRISPR first identified in E. coli. The term "CRISPR" was introduced in 2002 [15]. CRISPR is greatly developing in recent times it is majorly useful for life sciences [16]. The recent advancements of the CRISPR-Cas9 system are very useful as well as easy-to-used editing techniques They play great importance in the research area [17]. CRISPR-Cas9 is the newly developed genome-editing system. CRISPR-Cas9 technology is the latest genetic manipulation technique, it is recently used all over the world. The enzymes that act on DNA are crucial for genetic manipulation technologies [18]. The CRISPR system provides adaptive immunity to bacteria including archaea [19]. The CRISPR/Cas9 system involved two important components like Cas9 including sgRNA. Cas9 is obtained from Streptococcus pyogenes. Cas9 involved two domains as HNH domain including a RucV-like domain. Synthetic sgRNA with a length of about 100 nt [20]. CRISPR systems are majorly divided into two main classes. Class I consist of (type I, III, I) with class II including (type II, V, VI) [21]. The Class I CRISPR-Cas systems included multisubunit complexes. The class II systems involved a single Cas protein [22]. CRISPR-Cas9 system was obtained from type II systems [23]. Type II CRISPR/Cas9 systems are relatively simplest, they are studied properly and are majorly useful for genetic engineering. The sgRNA including CRISPR-associated (Cas-9) proteins are two important

components of the CRISPR/Cas9 system [24 ,27]. Studies that invented enzymes related to the CRISPR family, involved enzymes it encoded Cas genes smaller than SpCas9 (4.2 kb), such as SaCas9 (3.2 kb), NmCas9 (3.2 kb) including St1Cas9 (3.4 kb). These enzymes assist in the packaging of viral vectors. The newly identified enzyme called Cpf1 and shorter crRNA sequences uses an alternative for SpCas9. Moreover, newly developed C2c2 (Cas13a) including C2c6 (Cas13b) it degrades RNA [25]. Newly, CRMAGE that combines to CRISPR/Cas9 system including MAGE technique, is established it allows effective as well as fast genome engineering. In an earlier study, the CRMAGE technology used, recombineering efficiency extended by around 90% including protein synthesis modulation efficiency extended by 64%. The newly, identified CRISPR-Cas13 is the capability to knock down RNAs use of RNA-guided RNA-targeting CRISPR-Cas effector Cas13a from Leptotrichia wades (LwaCas13a) although most effective in an E. coli [26].

CONCLUSION

Genetic engineering is used in the formation of transgenic organisms it is the new main advancement in agriculture, medical sciences as well as biotechnological applications. This technique is mainly useful for human purposes. This is a great time its awareness with the education of genetic engineering is assisting. The researchers are generally to coordinate with discussing development in the field of Genetic engineering. The fast advancement in CRISPR/Cas9 system greatly revolved around genetic engineering, particularly modifications of the Cas9 as well as sgRNA to attain proper editing.

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