Biological Forum – An International Journal **10**(1): 158-167(2018)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Proteases: Industrial Applications and Approaches used in Strain Improvement

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(Received 10 March 2018, Accepted 27 May, 2018)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Proteolytic enzymes are the enzymes that bring about degradation of the proteins into peptides and amino acids. They are obtained from many sources such as plants, animals, micro-organisms. The enzymes isolated from micro-organisms are more preferred source and less costly as compare to the enzymes obtained from the plants and animals. Proteases are now widely used in pharmaceutical, leather, industrial waste management, brewing industry, food industry and providing economic benefits. Due to rapid increase in the applications of proteases there is continuous demand in both qualitative and quantitative enhancement of enzyme through strain improvement by inducing genetic variation. Enhanced production of enzymes and subsequent screening of parent and mutated strain through systemic mutagenesis is involved in strain improvement. Many physical and chemical mutagenic agents are generally used for yield improvement. This review will highlight the overview on classification, applications and various techniques used in strain improvement.

Keywords: Proteases, Classification, Sources, Physical and Chemical mutagenesis

INTRODUCTION

Proteases are the enzymes which hydrolyse the peptide bonds through which amino acids are linked together in the polypeptide chain (Gupta and Khare, 2007). Proteases represent about 60% of the total worldwide sales of the enzyme (Vaishalakshi and Dayanand, 2009). Proteases are classified into six groups based on their catalytic activity and the presence of amino acid residues at the active site: aspartate, cysteine, glutamate, metallo, serine and threonine (Lopez-Otin and Bond, 2008). These enzymes are widely found in plants, animals as well as in microorganisms (Li *et al*., 2012).

Proteases now known for their many physiological and commercial applications and also perform various other functions too. They are widely used in biological processes, in regulation of metabolism and digestion of dietary proteins to allow absorption of amino acids (Choi *et al*., 2015). These enzymes carried out various other processes involving maturation of prohormones, blood coagulation, apoptosis, immune function, and the recycling of cellular proteins (Gupta and Khare, 2007). Proteases also have various industrial applications as they can be widely used as a biochemical reagent and also used for manufacturing of various products. Proteases are utilised in various food processing applications. In cheese making processing, chymosin performs hydrolyzation of the specific peptide bond (the Phe-15-Met 106 bond) and generate park-k-casein and macro peptides (Smit *et al*., 2005). They also find their application in pharmaceutical and leather tanning industries (Saeki *et al.,* 2007). Beside this, they are also used in preparing soy sauce, other hydrolyzates and for meat tenderization (Wang and Wang, 2004). Due to rapidly increasing demand of these enzymes in various industrial applications, manufactures are always looking for various strain improvement techniques to improve the production, stability and specificity of proteases (Li *et al*., 2012).

TYPES OF PROTEASES

Proteases are classified on the basis of site of action on the polypeptide chain into two categories: exopeptidases and endopeptidases.

Exopeptidase

The exopeptidases catalyses and cleaves the terminal peptide bonds near to the amino and carboxy termini of the substrate i.e. they act only at the end of polypeptide chain (Rao *et al*., 1998). Exopeptidase and endopeptidase are further classified as aminopeptidase and carboxypeptidase. Aminopeptidase is a protease enzyme that performs single amino acid cleavage from the amino terminal end of the peptide (Motyan *et al*., 2013). These enzyme act on free N terminus of a polypeptide chain and cleave a single amino acid residue. On the basis of their action at neutral or acidic side chains they are further classified as aminopeptidase N and amionopeptidase A respectively (Motyan *et al*., 2013).

Aminopeptidase-1 is a largest protease produced from *Escherichia coli* with 400 kDa in molecular weight Fig. 1 (Vaishalakshi and Dayanand, 2009; Rao *et al*., 1998).

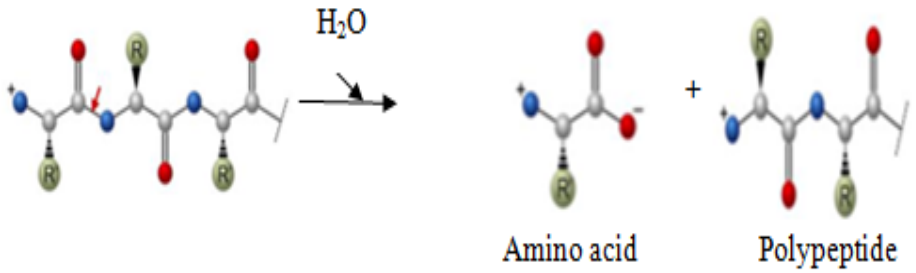

Fig. 1. Reactions catalysed by aminopeptidases (Motyan *et al*., 2013).

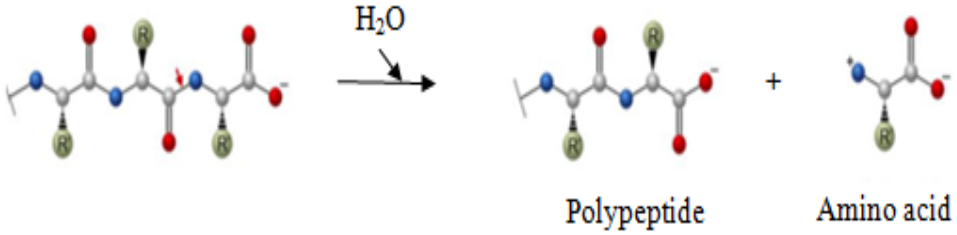

Fig. 2. Reactions catalysed by carboxypeptidases (Motyan *et al*., 2013).

On the other hand carboxypeptidase is an enzyme cleaving amino acid at the carboxyl terminus of peptide chain (C terminal) Fig. 2.

Carboxypeptidases are found in animal, plants and humans (Vaishalakshi and Dayanand, 2009). The several functions of carboxypeptidases include: help in digestion of food, mature proteins, helps in blood clotting, regulate biological process, growth factor production, (Motyan *et al*., 2013). They are further classified on the basis of nature of amino acid residues at the active site of the enzymes carboxypeptidases are classified further and comes under major groups metallo carboxypeptidases, serine carboxypeptidases and cysteine carboxypeptidases (Rao *et al*., 1998). Enzymes that use serine residues in the active site are called serine-carboxypeptidases and are mainly isolated from *Saccharomyces* sp., *Penicillium* sp. and *Aspergillus* sp. (Vaishalakshi and Dayanand, 2009). They shows similar substrate specificities, but difference in the activity by varying physiochemical properties like pH optimum, optimum temperature, stability, molecular weight, and effect of inhibitor. Enzymes utilizing metal ions in at their active site are named as metallo carboxypeptidases and mainly isolated from *Saccharomyces* sp. and *Pseudomonas* sp. (Motyan *et al*., 2013). These carboxypeptidases require Zn^{+2} or Co^{+2} to enhance their activity (Browner *et al*., 1995). On the other hand enzymes with utilising cysteine in the active site are called cysteine carboxy-peptidases. These enzymes also referred as thiol proteases are found in virus, bacteria, protozoa, plants and mammals and recently these are also found to be present in fungi (Browner *et al*., 1995).

Endopeptidases are the peptidases that break peptide bonds of non-terminal amino acids i.e, away from N and C terminus as shown in Fig. 3. They break within the molecule (Motyan *et al*., 2013)**.**

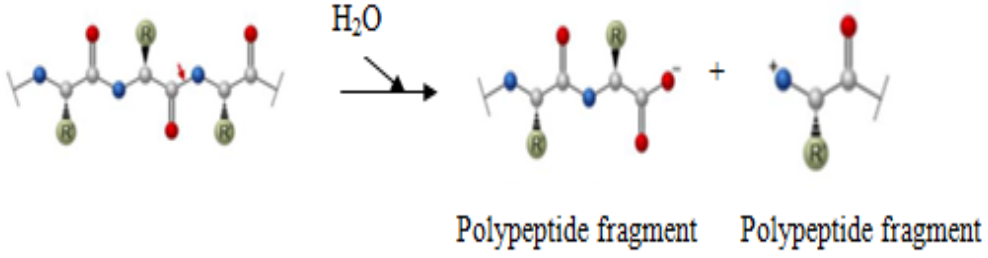

Fig. 3. Reaction catalysed by endopeptidases (Motyan *et al*., 2013,).

The endopeptidases are further classified as serine proteases, cysteine proteases, metalloproteases and aspartic proteases on the basis of their catalytic mechanism as shown in Table 1.

Table 1: Classification of proteases on the basis of amino acid residue at the active site.

Class	Mechanism	Example	References
Serine proteases	Serine residues in active site.	Trypsin, chymotrypsin,	Motyan *et al*., 2013
Metallo proteases	Metal ion in the catalytic core	Thermolysin	Browner *et al.*, 1995
Cysteine proteases	Carboxypeptidase uses cysteine in active site.	Bromelain, papain	Chapman *et al*., 1997
Aspartic proteases	Two aspartic acid residue bonded with activated water in network fashion in the active site.	Pepsin, renin	Tang and Wang,1987

DIFFERENT SOURCES OF PROTEASES

The proteases are necessary for all living organisms and for their metabolism. They are in numerous sources of proteases like animals, plants and microorganisms (Rao *et al*., 1998)**.** Plants producing proteases are keratinases, papain, bromelain (Schechler and Burger, 1967; Motyan *et al*., 2013). Proteases namely 'Camein' and 'Milin' obtained from latex of *Ipomoea carnea* and *Euphorbia milii* respectively and are beneficial in various biotechnology industries (Rao *et al*., 1998). On the other hand various proteases namely pancreatic trypsin, pepsin, chymotrypsin, and rennin are animal proteases (Motyan *et al.*, 2013). Trypsin which is a serine protease hydrolyses food proteins into smaller units and is main intestinal digestive enzyme.

The proteases produced by microorganisms are more in demand in industrial applications as compare to the enzymes isolated from plants and animals (Bhunia *et al*., 2013). Microbes are preferred as a source of these enzyme due to their rapid growth with limited space required for their cultivation at industrial scale (Souza *et al*., 2015). Microbial enzymes shows wide range of physical and chemical characteristic and can be easily manipulated genetically in order to get desired enzymes(Gupta *et al.*, 2002). Also, another advantage is that these enzymes are produced at very low cost (Chanalia *et al.*, 2011).The limitations of the plant and animal sources to meet the desired characteristics for biotechnological applications has lead to the increase in demand of proteases from microbial sources (Gupta *et al.*, 2002). The microbial proteases today represent the 60% of the total worldwide market of the industrial enzyme (Souza *et al*., 2015).

PHYSIOLOGICAL FUNCTIONS OF PROTEASES

Most types of proteases are found in intracellular and extracellular space in all types of cell. The intracellular proteases exhibits some of the major function like inactivation and proteolysis of proteins, regulation of synthesis (Bond and Butler, 1987). Extracellular proteases helps in blood coagulation and in complement cascade event. Proteases are now involved in digestion, tumour growth and metastasis, in blood coagulation, tissue arrangement, cell growth and migration, morphogenesis in development, inflammation, release of hormones and pharmacologically active peptides from precursor proteins, transport of secretary proteins across membranes and also involved in many drug development (Leipner and Saller, 2001).

COMMERCIAL APPLICATION

All proteolytic enzymes show specific properties with regard to temperature, pH, ion requirement, specificity, activity and stability. These characteristics determine the application of protease in industries like food, leather, detergent and pharmaceutical (Jisha *et al.*, 2013; Shah *et al*., 2014). They are now also responsible for management of wastes from domestic and industrial activities. Some of applications of proteases are elaborated in Table 2.

A. Detergent Industry

Detergents are widely used for household laundry or as a reagent for cleaning contact lenses. Proteases now have a major role in all kinds of detergents and the detergent prepared from these enzymes are helpful in washing (Anwar and Saleemuddin, 1997). These enzymes also help in removing the stains such as of blood, proteins secreted from our own body and food such as milk, egg, meat, fish etc. (Kumar *et al*., 2008; Gupta *et al.*, 2002). Due to ionic strength of these proteases, they are best considered in detergent (Kumar *et al*., 2008). Proteases are considered best if it's ionic strength coincides with the pH of detergent solution and therefore increases the effectiveness of the detergent (Shah *et al*., 2014; Kumar *et al*., 2008). Protease extracted from *Spilosoma oblique* are widely used for removal of blood stains (Jisha *et al.*, 2013; Kumar *et al*., 2008). Alkaline proteases are used more with commercial detergents as these proteases are found to be more stable at various ranges of temperature and pH and removes stains more efficiently from clothes (Kumar *et al*., 2008; Anwar and Saleemuddin, 1997).

Serine proteases produced by *Bacillus* strains are also in great demand in the detergents market (Rao *et al*., 1998).

B. Leather and Wool Industry

Chemical methods which are used for leather processing lead to the environmental pollution and also affects the lives of living organism. Now chemical processing of leather has been overcome by the proteases enzyme (Shah *et al*., 2014). Traditional methods forremoval of hair and unwanted adhering of chemicals to **s**ubcutaneous layer causes a problem (Rao *et al*., 1998). The main problem with use of chemicals is caused is smell which cause suffocation and also the release of some unpleasant and harmful gases which can even cause death (Choudhary *et al*., 2004). These proteases used in leather industry are eco-friendly and act as an alternative to chemicals. The use of enzyme lead to the reduction of pollution and also increases the quality of the leather (Shah *et al*., 2014). The process of using alkaline proteases is more safe and pleasant as compared to the various reagents used in processing such as sodium sulphide, sodium hydroxide and also saves energy and time (Puvankrishnan and Dhar, 1986). Protease enzymes are also used in the wool and fabric industry. Wool is mainly composed of polypeptides and alkaline proteases are mainly used during wool processing (Shah *et al*., 2014). Earlier the chemicals were used in fabric industry for the finishing and shining of fibre and have many disadvantages. The main disadvantage is pollution created by these chemicals on the natural environments (Shah *et al*., 2014). The use of enzymes have replaced the chemicals from wool industries due to their certain advantages i.e. eco-friendly characteristics, easy isolation from the micro-organisms (Paranthaman *et al.*, 2009). The enzymes can be used for various processes such as polishing of wool fibre and desizing of cotton fibre etc. the enzymes are isolated from the micro-organisms so they are bio-degradable and thus create less pollution and also reduces time of processing (Srilakshmi *et al.*, 2014).

C. Food Industry

In food industry proteases are involved for various processes like baking, cheese making, for meat tenderization, preparation of soy hydrolysates, (Srilakshmi *et al.*, 2014). Proteases are now becoming important component in our food and help in improving the nutritional value, digestibility, flavour and minimizes the allergenic compounds (Odetallah *et al*., 2005). Proteases are used in soy sauce production which in result increases the yield in good amount and nutritional value. Soy proteins on treatment with alkalase at pH 8 results in soluble hydrolysates with good protein yield and low bitterness (Srilakshmi *et al.*, 2014).

Proteases are also greatly used nowadays to improve the meat tenderness or meat quality (Paranthaman *et al.*, 2009). Proteases are also used in the baking industry to quickly prepare the dough as its gluten is partially hydrolysed by a heat-labile fungal protease and it also reduces the viscosity of dough (Shah *et al*., 2014). For preparing biscuits weak-gluten flour is required and proteases play a major role in its preparation (Souza *et al*., 2015). In dairy industry the main role of protease enzymes is the production of cheese by hydrolyzing, specific peptide bonds to produce casein and macropeptides (Paranthaman *et al.*, 2009). They also play a major role in production of yogurt and other dairy products. Some proteases are also used to change and improve the colour and texture of some dairy products (Shah *et al*., 2014).

D. Brewing Industry

Proteases are also used in brewing industry to remove the haziness in brewing industry (Nelson and Youno, 1987). Due to the presence of certain proteins in the beer, it looks little hazy at freezing temperature and also affect the shelf life of a beer. So removal of these proteins from the beer helps it to look clearer (Paranthaman *et al.*, 2009). Maximum beverages are made with the process of fermentation with the use of various enzymes. Protease enzymes are added during the process of fermentation and *Bacillus subtilis* protease is used to solubilize protein from barley adjuncts (Nelson and Youno, 1987). Addition of proteases enzyme also helps in preventing the formation of precipitates when kept in cold temperature.

E. Pharmaceutical Industry

Proteases possess its various application as a distinct therapeutic class with diverse other applications. These are helpful in developing various drugs against fatal diseases like anticancer, antimicrobial, anti-inflammatory infections and dissolving clots (Srilakshmi *et al*., 2014). Now a days proteases are useful in treatment of cystic fibrosis, sepsis, digestive disorders, retinal disorder, and many more disease. Beside this they are also help in reducing the discomfort of breast engorgement in lactating women (Chanalia *et al*., 2011).

A protease known as Serratio peptidase produced by *Serratia* species play a major role against inflammation and is also effective in curing pain (Vaisar *et al*., 2007). Asparaginase from *Escherichia coli* and collagenase also play a major role in removing asparagine from the blood in forms of lymphocytic leukemia, burns and wounds respectively (Chanalia *et al*., 2011).

Nattokinase from *Bacillus subtilis* is also helpful against cardiovascular disease. Trypsin was eliminated by alkaline proteases *Conidiobolus coronatus* (Chiplonkar *et al*., 1985). Alkaline proteases produced from *Bacillus* sp. CK-114 also act as a thrombolytic agent due to its fibrinolytic activity (Kim *et al*., 1996).

F. Photographic Industry

Alkaline proteases play a major role in photographic industry as these enzymes helps to recover silver from the used X- ray and photographic films. These used films have gelatin layer which contain 1.5%-2% of silver by weight. The alkaline proteases from *Bacillus coagulans* PB-77 (Gajju *et al.*, 1996), *Bacillus* sp. APR-4 (Kumar *et al*., 2008) are useful in decomposing gelatinous coating on used films from which silver can be recovered (Shankar *et al*., 2010).

G. Management of Industrial Waste

Proteases are also used for the discharge and management of industrial waste. Enzymatic methods are used to treat the different types of waste such as solid, liquid and hazardous waste (Shankar *et al*., 2010). The protease enzymes are helpful in degradation of waste by transformation to other products or some valuable products. In poultry industry, the chicken feather is composed of over 90% proteins, as keratin the main component in this waste is an insoluble protein (Shankar *et al*., 2010). This waste is decomposed by the pre-treatment with NaOH, mechanical disintegration, and enzymatic hydrolysis and results in complete solubilization of feather (Deivasigamani and Alagappan, 2008). Some proteolytic enzymes from *Bacillus subtilis*, *Bacillus amyloliquefaciens* and *Streptomyces* sp. and a disulphide reducing agent such as thioglycolate plays a major role in hair degradation and are also involved in clearing pipes which are clogged with hair containing deposits (Atalo and Gashe, 1993). Leather industry waste are also degraded and transformed into useful products like animal glue, protein concentrate for fodder (Dalev and Simenova, 1992).

H. Degumming of Silk

A proteinaceous component sericin constitutes about 20-30% of raw silk and it mainly involved in wrapping fibroin (Najafi and Deobagkar, 2005). Due to the presence of sericin the quality of silk fibres are rough but after its removal silk fibres turns very soft (Chopra *et al.*, 1994). Traditionally the degumming of silk was done with the soap or alkali. The use of soap or alkali have some disadvantages as it is a crude treatment and also these chemicals are not eco-friendly and proteases are used as an alternative to these chemicals (Najafi and Deobagkar, 2005). Trypsin, papain and bacterial enzymes are used mainly for degumming process. These enzymes increases the strength of the yarn and does not causes environmental pollution therefore proteases are greatly used nowadays for degumming of silk (Chopra *et al.*, 1994).

Table 2: Applications of proteases in different industries.

Industries	Application	Enzymes	Reference
Food	Improved digestibility, solubility flavor, palatability and viscoelsatic properties; reduced allergenicity	Alkaline proteases	Rao *et al*., 1998
Detergent	Improved washing	Alkaline proteases	Gupta *et al.*, 2002
Meat and fish	Meat tenderization	Papain, other proteases	Rao *et al*., 1998
Baking	Dough conditioner	Neutral proteases	Souza *et al*., 2015
Leather	Leather processing: Dehairing, bating, tanning	Trypsin, alkaline protease	Choudhary *et al*., 2004
Bioremediation	Waste treatment	Alkaline proteases	Ramnani *et al*., 2010
pharmaceuticals	Anticancer, anti-inflammatory, clot-buster agents	Trypsin	Srilakshmi*et al*., 2014
Bevarages	Removes turbidity, chill proffing	Papain	Paranthaman *et al*., 2009
Photography	Silver recovery from used X- ray and photographic films	Several proteases	Shankar *et al*., 2010
Bioactive peptide synthesis	Synthesis of bioactive peptides	Alkaline proteases	Kumar *et al.*, 2003

STRAIN IMPROVEMENT

Despite the intensive use of enzymes in biotechnological applications, there is still significant need for improvement of enzymes in industrial processes (Sher *et al*., 2012). Most of the industrial strains currently used for the production of novel compounds are generally obtained from their natural habitats (Huang *et al.*, 2007). So they are unable to synthesize desired products in bulk and it affects both cost and productivity.

So to meet this continuous world wide demand of enzymes, it become important for selection and development of strains with novel properties (Sher *et al*., 2012). Improved strains can reduce the costs with increased productivity along with desirable characteristics (Jeyasanta and Patterson, 2014). Mutation brings about alteration in the sequence of genes controlling the specific characters to improve the microbial strains for overproduction of the desired product (Sher *et al*., 2012). Mutation is the primary source of all genetic variation and has been extensively used for enhanced enzyme production for industrial use (Gupta and Ramnani, 2006).

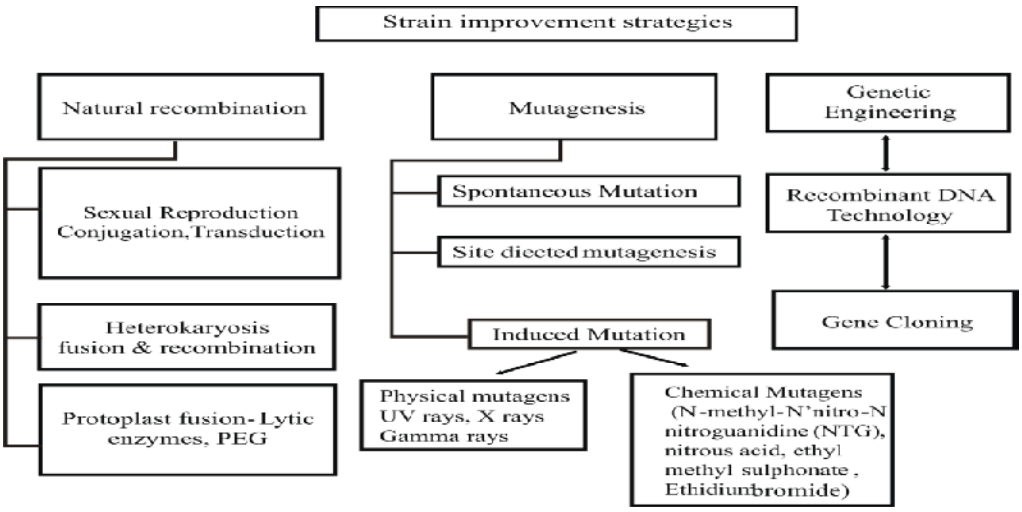

Fig. 4. Various techniques used for strain improvement with microorganisms (Pathak *et al*., 2015).

There are different approaches to improve the wild strain in term of specific characteristics like yield, efficiency, stability and specificity Fig. 4. It has been achieved by genetic engineering, natural recombination, through classical approach by random mutagenesis using physical and chemical methods of mutagenesis (Soliman *et al.,* 2004). Classical genetic approach of mutagenesis involves the improvement of the desired metabolite yield in a particular wild strain using random mutations (Cheng *et al.*, 2008)

RANDOM MUTAGENESIS

Classical approach by random mutagenesis is ideal method of choice for short-term strain development is frequently the (Huang *et al.,* 2007). Parent strain is subjected to different physical and chemical mutagens that bring about alterations at the gene level in a microorganism (Sher *et al*., 2012). A series of different mutagenic treatments using either chemical mutagens like Ethylmethanesulfonate (EMS), Methylmethanesulfonate (MMS), Ethidiumbromide (EtBr) or physical mutagens like UV radiations, X-rays are used with parent strain for developing a better yielding strain (Sher *et al*., 2012).

Different mutagens possess mechanisms of action involving base transitions, base additions and base deletions (Soliman *et al.,* 2004). Mutation depends upon minimum dose required and time of exposure for mutation of particular strain which is a lethal dose at which survival rate of colonies remain 50% in parent strain and survived colonies show mutation (Sher *et al*., 2012). In case of "under mutagenesis" no genetic alteration occur in the parent strain and mutant survivors remain identical to parent strain (Huang *et al.,* 2007). On the other hand, "Over mutagenesis" produces population of mutant survivors that accumulate multiple mutations and in turn significantly reduces the chance of desired mutation (Parekh *et al*., 2000).

Mutagenesis involves the deletions, insertions or additions of nucleotide which results in the changes in their properties.

A. Spontaneous mutation

In spontaneous mutation, gene is modified unintentionally during the life cycle of an enzyme. But the spontaneous mutation rate is very slow which is further increased by the use of various mutagenic agents (Agrawal *et al*., 1999**).**

B. Induced mutation

In induced mutation, gene is modified intentionally by various physical and chemical mutagens. Physical mutagens includes UV rays, gamma rays and X rays in which UV rays is more preferred mutagen (Agrawal *et al*., 1999). Effect of various mutagens are shown in Table 3.

Table 3: Effect of mutagen on genes of microorganisms.

Mutagen	Mutation involved	Effect on DNA
X-rays	Single or double stranded DNA breakage	Deletions
UV radiations	Pyrimidine diamerization	AT-GC,GC-AT transition
5-Chlorouracil	Results in faulty pairing	AT-GC,GC-AT transition
Hydroxylamine	Deamination of cytosine	GC-AT transition
Nitrous acid	Deamination of A,C and G	Bidirectional transition
Ethylmethanesulfonate	Alkylation of bases C and A	GC-AT transition
Ethidiumbromide	Intercalation between two base pairs	Frame shift, microdeletions

References: (Shibutani *et al*., 1991; Bockrath *et al*.,1987)

Chemical mutagens involves nitrous acid, N-methyl-N'nitro-N nitroguanidine (NTG), ethyl methyl sulphonate, ethidium bromide. UV treatment commonly induces deletions, transitions, transversions and frame shift mutations and is used for the productions of industrial enzymes like proteases in mutants with enhanced activity and resistance to the environment. With the use of UV irradiation the phenotypic mutants of *Bacilllus* strain RS were developed with increased protease production and stability to the wide range of temperature, pH and resistance to different inhibitors (Karn and Karn, 2014). UV mutagenesis and gamma mutagenesis of *Pantoea dispersa* producing chitinolytic enzyme was done for strain improvement. The isolated mutant of *Pantoea dispersa* resulted into increases in activity of proteolytic enzyme (Gohel *et al*., 2010). Physical mutagen like UV irradiation and chemical mutagens like ethidium bromide and ethylmythyl sulphonate were used with *Bacillus Cereus* GD 55 for strain improvement and showed the higher productivity of fibrinolytic protease (Raju and Divakar, 2013).

C. Site directed mutagenesis

It is the most popular and important technique mainly used for introducing mutation in the DNA of particular organism. Site directed mutagenesis is more complicated as compared to random mutagenesis (Estell *et al.,* 1985). In this approach, it is very necessary to have information about mechanism, knowledge of sequence and its structure (Karn and Karn, 2014). The alteration of specific base pairs may or may not lead in disruption of other characteristics (Burg *et al*., 1999). It is more preferred technique as it can give us the product with desired properties (Estell *et al.,*1985).

In recent studies, mutagenesis is combined with the genetic engineering for the modification of isolated genes or parts of isolated genes to get desired product (Hutchison *et al.,* 1978). The desired change in particular sequence resulted in increased thermostability, altered substrate range, altered pH range etc. (Estell *et al.,* 1985). Thermolysin like proteases obtained from *Bacillus stearothermophilus* were modified using site directed mutagenesis technique which further resulted in increase in its stability, and better resistant to temperature and pH (Burg *et al*., 1999). As the industrial use of thermostable and alkaline proteases are expected to grow tremendously in the coming decade so, industries always keep in searching for new advanced and cheaper methods for overprotease production (Vanitha *et al*., 2014). Examples of some proteases engineered for industrial applications are given in Table 4.

Table 4: Examples of protease engineering for industrial use.

Sources	Nature of proteases	Advantage	Modification	References
Bacillus subtilis	Subtilisin	Enhanced stability in organic solvent	Disulfide bond modification	Takagi *et al*., 2000
Bacillus amyloliquefaciens	Alkaline protease	pH modification	Aspartic acid replace with serine at position 99	Thomas *et al*., 1985
Bacillus stearohermophilus	Neutral protease	Enhanced thermal stability	Aspargine replaced with leucine at position 241	Eljsink *et al*., 1991

FUTURE PROSPECTS

Protease is extensively used in various industries and its significant role has led researchers to focus on regulatory, biomedical and microbial aspects of the protease enzyme. Various microbial sources already play an important role in the production of enzymes and application of various microbial alkaline proteases is expected to increase in the future. Protein engineering and gene cloning is playing a major role in developing the enzyme with various new functions and in future the potential of enzymes will be in great demand.

So in future, with the command on function and structure of proteases and in vitro changing the primary structure of proteases using protein engineering, proteases will be produced with desired applications.

CONCLUSION

The demand of protease enzyme gaining day by day at commercial, industrial, pharmaceutical, analytical, diagnostic sectors. They occur ubiquitously in, animals, plants and microorganisms. As the enzymes obtained from the animal and plants are not much effective and costly so the enzymes produced with the help of micro-organisms are mostly preferred due to their rapid growth, and with limited space requirement for cultivation. Protease enzymes are utilised in food industries, fed industries, pharmaceutical industries, leather industries. Proteases are also a great source for management of waste. Their degradative nature also increases their useful for protein digestion in tissue dissociation, cell isolation and in cell culturing. Due to extensive use of protease enzyme at various industrial applications, strain improvement brings about hyper production of enzyme which ultimately leads to the reduced production cost of enzyme.

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