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Intervention in Molecular Biology to Increase Enzyme Activity and Produce Commercial Enzymes

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Abstract

Metagenomics and directed evolution have changed how we look for new enzymes in harsh settings and how we change current enzymes to make them do what we want. Researchers can make enzymes with more activity, stability, and substrate selectivity using next-generation sequencing, site-directed mutagenesis, fusion protein, surface display, and other molecular biology techniques. These enzymes are used in industry. Many large-scale industrial enzyme processes still need to get around the limits of catalytic process activity. This piece talks about how enzymes are used in industry and how metabolic engineering is being used to make enzymes work better and produce more.

Keywords: Enzyme; Molecular biology; Metagenomics;

Introduction

The change in the chemistry business is making it harder to make chemicals with microbes and enzymes. To switch from petrochemicals to green chemistry, we must avoid getting stuck when we look for new enzymes, make biocatalysts, and design fake enzymes. Non-natural chemical processes can be sped up by enzymes for each natural molecule. These enzymes change in vivo to digest new manmade chemicals [1]. Enzymes are used in many businesses,

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such as food and drink (amylase, lipase, pectinase), textiles and leather (laccase, cutinase, protease), cosmetics (SOD, oxidase), pharmaceuticals (chitinase, streptokinase), chemicals (epimerase, lipase), and others.Enzymes react under normal temperatures, pressures, healthy pH, in watery and organic fluids. Also, surface functional groups do not have to be activated or reactivated at active sites for enzyme-mediated processes to happen. These enzymes are selective, enantioselective, and not good at breaking down substrates that are not natural. Few industrial processes need catalysts that can handle high temps and pressures, but these enzymes lose their specific activity, which lowers the end-product yield and efficiency. When natural enzymes don't work, scientists have to come up with new ones [2]. Metagenomics is now used to figure out the species of microorganisms and find new enzymes[3]. Metagenomic methods have been used to find endoglucanase in the gut of a termite, -amylase in sheep, and esterase in the sludge of a lotus pond, among other things. Using genetic engineering, genes from bacteria that have never been grown in a lab, are dangerous, or grow slowly can be copied into GRAS germs to make them more productive. Even though scientists are looking for new places for enzymes to live, their low activity, stability, and precision limit their use in industry. Enzymes have been made better by using guided evolution, fusion proteins, surface display, and fake amino acids to get around these limits. Researchers could make new enzymes based on the link between the shape and function of a gene's code.

Enzymes are very important for everyday life and business. Researchers can now use bioengineering and molecular biology to make new enzymes with better and more unique functions. This piece gives an update on how enzymes are used in industry and how molecular biology is used to find new enzymes and improve existing ones to make them more active and stable [4].

Industrial enzymes

Microorganisms and enzymes have always been used to make food and drinks. Enzymes have been used for many years in the food, feed, cloth, leather, cosmetic, drug, and fuel businesses. This is possible because of improvements in microbe culture, high-throughput screening, and fermentation technology. Bacteria and fungi make most enzymes because they grow quickly, don't need much food, and are easy to grow. Almost 75% of the enzyme market is made up of hydrolases (protease, lipase, amylase, and cellulases), and demand is

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growing [5]. In hydrolytic processes, enzymes are often used to make food more soluble and clear. Trends in the food business are functional foods (like prebiotics and probiotics) and fake sugars. Textile threads need to be sized, bleached, dyed, and given functions. Energy and chemicals are needed for every step. Textile enzymes can shrink fabrics, keep them from getting wrinkles, and improve other qualities [6]. Chemicals made from petrochemicals are used in cosmetics. Customers want natural cosmetics, so companies are looking for enzymes that can be used in makeup. Researchers have looked into using superoxide dismutase (SOD), protease, and lipase in cosmetics.

Enzyme	**Function**	**Major applications**
L-asparaginase	Hydrolyze L-asparagine	Acute lymphoblastic leukemia therapy
Streptokinase	Promote lysis of Arg/Val bond in plasminogen	Myocardial infarction clot breakdown.
Mannase	Degrade mannan	Mannan stain eradication.
Lipase		Decompose oil-related stain-causing fats.
Nattokinase		For inflammatory or fibrotic disorders..

Table 1; Lists the various enzymes' industrial uses

Enzyme manufacturing through molecular biology

In most businesses, biocatalysts like enzymes speed up biological and chemical processes. For industrial enzyme production, the best place to get enzymes is from microorganisms, which can be grown quickly in fermenters. Metagenomics extracts unique enzymes from uncultured microbes. Directed evolution promoter screening, engineering of translation and transcription factors, codon optimization, protein fusion, and other techniques can be used to change or design enzymes for commercial use [7].

Metagenomics and enzyme discovery

Metagenomics, which is also called population genomics, community genomics, and environmental genomics, looks at the structure of the genomes of microbial communities in different environments. Without raising the microbe, the gene of interest can be found

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through direct sequencing. To make metagenomic libraries, the right vectors are used to separate gDNA from external materials. Several sizes of inserts have been looked into. Some examples are phage, BAC, cosmids, and fosmids. To make the vectors, Escherichia coli, Bacillus subtilis, Pseudomonas, and other organisms are changed. This screening process finds new enzymes without using germs that can't be grown in a lab. Sequence-based metagenomics involves reading DNA from samples taken from the environment, putting together genomes, figuring out what genes do, looking for the whole metabolic pathway, and comparing animals from different groups. Next-generation sequencing (NGS) could give us a lot of information for a small part of what Sanger sequencing costs. NGS and current computer methods make it possible to analyze large amounts of genomic data, but gene labeling only rely on how similar the sequences of public database genes are. Because of this restriction, 40–50% of the genes in the genome are listed as "hypothetical." Functional metagenomics looks through metagenome libraries to find out how specific proteins work. Metagenomics can't tell which microbiome members are active and which are not. Putting together metagenomics, metatranscriptomics, and meta-proteomics could make it easier to screen and choose enzymes. Metatranscriptomics, also known as RNA sequencing (RNASeq), can be used to look for proteins that are being actively produced in a microbiome at a certain time and in a certain setting. Metaproteomics studies all environmental bacteria proteins at a specific time. Metaproteomics of extracellular proteins from natural samples of the environment is hard because they are hard to separate and focus from the extracellular matrix in a way that works every time. This is why most metaproteomics studies look at the proteins inside cells. Bioinformatics is used for things like genome analysis, Pfam analysis, predicting structures, and phylogeny [8].

Enzyme engineering

Metabolic engineering could help in the search for enzymes that can be used in industry. Genes decide how it looks, how it works, what it makes, and how it is controlled. So, tactics that change their DNA makeup have helped them make more of them. 3.2.1[9].

Orgin of promoters

Promoters are the first step in making enzymes through engineering. At the promoter area, RNA polymerase starts the process of transcription. For genetic production on a large scale, you need a strong promoter that is expressed all the time and has a high affinity. Sequencing

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and bioinformatics have made it possible to test promoters in organisms that are not models. It's important to do promoter engineering. This can be done by mutagenesis, making native promoters, or testing strong manufactured promoters. Mutations in the inulase promoter and signal peptide of Kluyveromyces marxianus boost the production of lignocellulolytic enzymes. To make more, use a strong promoter to clone the gene. Cbh1 is a strong promoter that makes T. ressei make more cellobiohydrolase II (cbhII). In another study, S. cerevisiae with native SOD1 and PSE1 under the control of the pgk1 promoter made more cellulase. Engineering methods that make sense also work. kasOp is a promoter of Streptomyces coelicolor. This promoter's ScbR2 and ScbR binding sites were taken away in two steps [10].

Enh At different stages, gene expression engineering is needed for making enzymes for industry. Explore translation methods. Translation processes use mRNA to make proteins and control how they fold, shape, and get out of the cell. To make more enzymes, all mRNAs must be turned into proteins and folded. Quantitative proteomics showed that 17 factors linked to translation make P. pastoris make more xylanase. Protein kinase is made too much of In modified species like P. pastoris, the production of phytase from a different gene is improved by the regulatory component Bcy1. Translation is controlled by Fhl1p and other transcription factors, which break down rRNA and copy ribosome proteins. Fhl1p makes both phytases and pectinases more active. Ribosome Binding Sites (RBS) are another good target for improving the speed of translation. Checking out RBS libraries may improve the flow of translation reactions. People have looked at E. coli and Synechocystis [12]. anced transcription

Translation-controlled translation

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Codon tuning makes translation stronger

Codon optimization is a way to change the way genes work to make more proteins without changing the amino acids. There are 64 codons and 20 amino acids in proteins. There are 61 codons that tell the cell how to make 20 amino acids. Three of these codons tell the cell to stop making proteins. Codon degeneracy allows 10207 sequences to create a 375-amino-acid protein. But animals prefer one identical codon over the other (called "codon bias"), which has a direct effect on how genes are expressed[13]. Most of the amino acid leucine in humans is marked by the CUG codon (39.5%), but only 11.1% of it is in S. cerevisiae (Sen et al., 2020). Codon optimization is a very important part of how genes are expressed in different hosts. 105 times more protein can be made when common and host-specific codons are used instead of rare ones. Codon frequency is being optimized by Codon planner, Gene Designer, and planner. The choice of host, the open reading frame sequence, and the upstream and downstream regulatory components all affect the production of recombinant proteins.

Engineering for catalytic action

To make enzymes more specific, stable, and selective, scientists use site-directed mutagenesis, directed evolution, linker-enzyme fusion, cell surface display technologies, and amino acids that are not found in nature [14].

Evolution with a plan

For business processes, biocatalysts are used instead of manufactured catalysts. These biological catalysts have high specific reaction rates and very enantioselective and regioselective reactions on substrates of interest. Methods used in business and the way our bodies work don't favor enzymes that form on their own[15]. Directed evolution improves the way enzymes work. Directed evolution, also called laboratory evolution, imitates natural selection by modifying enzymes so they can change and merge to make a recombinant enzyme with better properties faster. How can development be guided? Fig. 1a shows that random mutation, DNA rearrangement, selection, and high-throughput screening are used.

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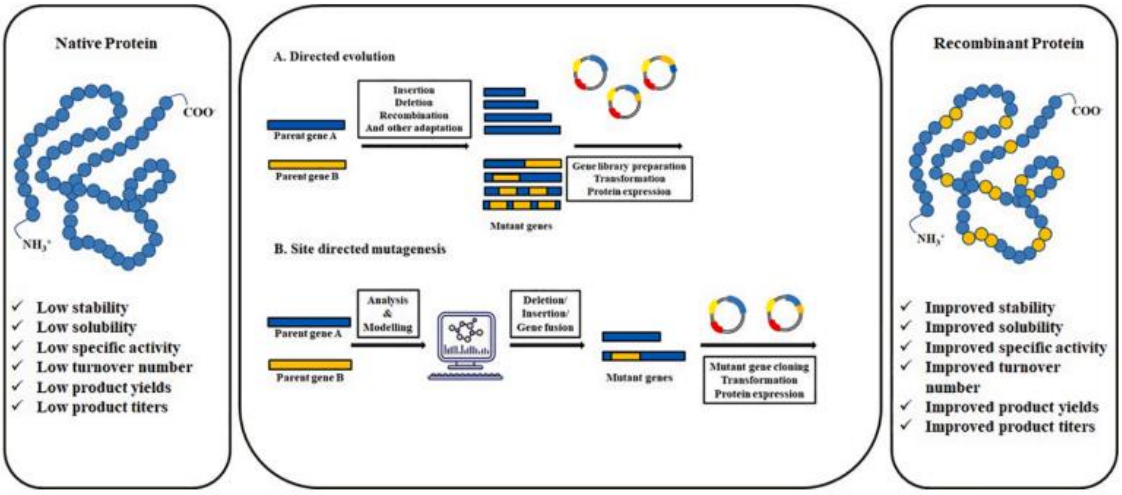

Fig.1 Directed evolution (A) and site-directed mutagenesis (B) enzyme engineering.

Site-directed mutagenesis (SDM) is a way to change the way a protein works by changing an amino acid at a particular spot (Fig. 1b). By adding or taking away a spot from enzymes with known sequences, recombinant proteins can be made that are more specific, stable, active, and soluble. Proteases and lipases that are sold in stores are better. SDM made natokinase work better and be more stable by two rounds. Harobin is a serine protease. Random mutation and SDM were used to improve its fibrinolytic and antithrombotic effects [16].

Protein linkers

Since the end product may not be made in a single step in natural or non-natural metabolic reactions, complex linked processes are stable, productive, have insufficient functional expression, and have a medium level of tolerance. By imitating nature's ability to bring enzymes together, we can get around the limits of industrial scale. With the help of new technology, two or more enzymes with different catalytic properties were joined together to make a hybrid or recombinant protein with better catalytic efficiency. 230 KDa Anaerocellum thermophilum cellulases are natural groups of enzymes that work as both endogluconases and exogluconases [17].

Unnatural amino acids

Canonical amino acids control complex cellular processes, bring in cofactors, and change after translation to change proteins and peptides. When cyclins are covalently bound to kinases, chaperons are covalently bound to HSP90, NAD is covalently bound to sirtuins, and AMP is covalently bound to AMP-activated kinase, Kcat/Km goes up. In the translation of

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mRNA, L-amino acids (aa) are used to make peptide chains, but few D-amino acids are found. The racemic D and L aas in the non-ribosomal, ribosomal, and post-translationally changed peptides are fixed by a few enzymes in the next step. For example, L-serine and L-threonine are changed into D-alanine and D-ethyl glycine by dehydratases and hydrogenases [18].

Technology for touch screens

In whole cell or enzyme-mediated biosynthesis, the main problems are getting the substrates to the right place and cleaning up the enzymes. Surface display technology (SDT) gets around these problems by putting proteins or peptides on the outside of a cell, where they can respond in the outside medium without the need for substrate transport. The transporter protein and the traveler protein are joined together. The signal peptide of the carrier lets the traveler get from the inner membrane to the outer membrane. Carrier proteins control traveller protein size and direction. A good signal peptide sends the traveler to the secretory route, where it joins up with the anchor or surface motif and tags the epitope so that it can be expressed and found [19].

Problems and opportunities

Most biological enzymes and enzymes that make things work come from bacteria. The enzyme business is the biggest in the world, and there is always a need for new enzymes with better activity, stability, and substrate specificity. Even with bioengineering, almost 99% of microorganisms are still unknown. To determine bacteria, you need to study the physical and biological factors of the world, and vice versa. Recent advances in metagenomics, genome mining, and high-throughput sequencing are making a lot of data that doesn't have much biological value [20]. Bioinformatics can take data from metagenomics and turn it into information about biology. The results of data analysis must be put together with what was already known. Along with these problems, study on new enzymes relies on the size and number of genes in metagenomic data, the availability of host vector systems, and the efficiency of screening systems. To find positive clones faster in hundreds of metagenomic libraries, we need new hosts, vectors that work on a wide range of hosts, and biosensors.

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Conclusions

Green chemistry is famous all over the world because processes that use enzymes make less waste. Molecular engineering methods like metagenomics and site-directed mutagenesis have been used to find and improve many enzymes. But lab-scale study only has a few uses, so the enzyme-based business needs methods that work on a larger size. Experimental and statistical methods for creating enzymes will lead to the discovery of new enzymes that can do more than one thing and have useful business uses.

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