MICROBIAL BIOTECHNOLOGY

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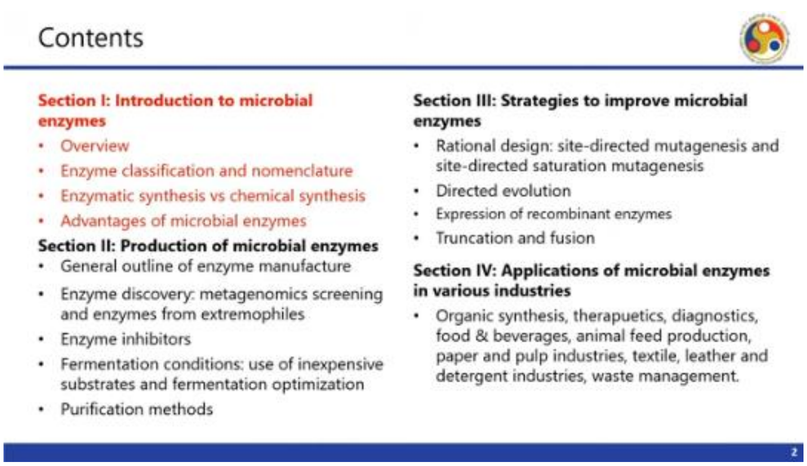
Lecture-21

Lec 21: Microbial Enzymes of Industrial Importance

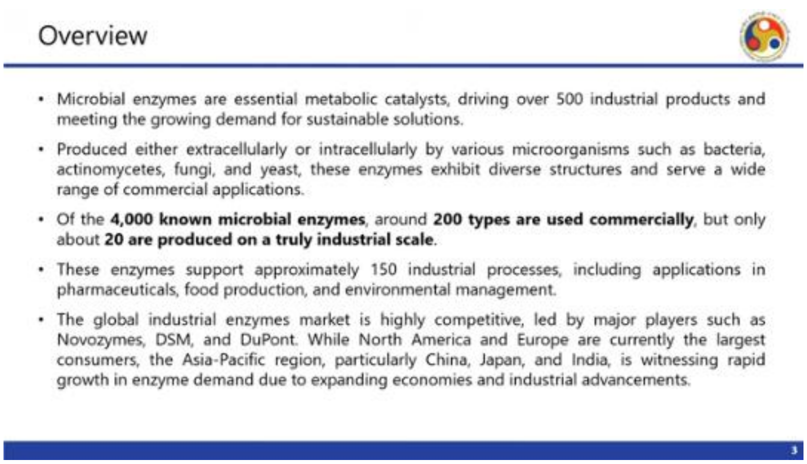
Hello friends, welcome to my course on microbial biotechnology. We are in module number 6, discussing industrial and pharmaceutical applications of microorganisms. Today, we will discuss microbial enzymes of industrial importance. The lecture is divided into three broad sections. In the first section, we will give an introduction and an overview. Then, we will cover enzyme classification and nomenclature.

Next, we will compare enzymatic synthesis versus chemical synthesis and discuss the advantages of microbial enzymes. In Section 2, we will discuss the production of microbial enzymes. In Section 3, we will discuss strategies to improve microbial enzymes. And in Section 4, we will discuss the applications of microbial enzymes in various industries. So, let us first have an overview of microbial enzymes in industry.



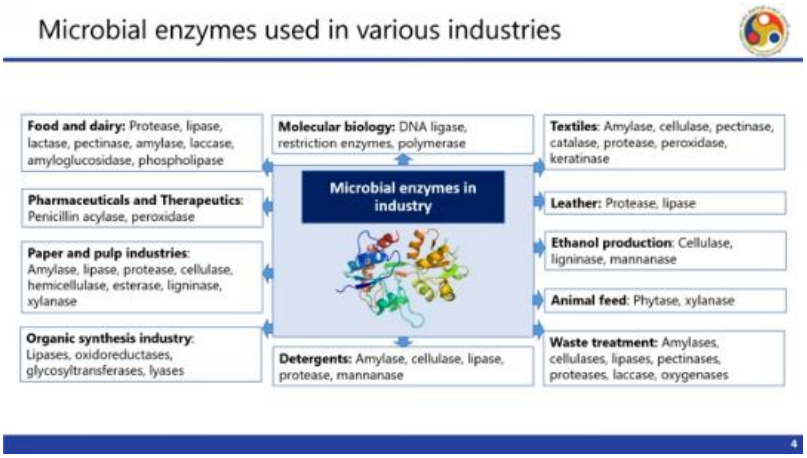
Microbial enzymes are essential metabolic catalysts, driving over 500 industrial products and meeting the growing demand for sustainable solutions. Produced either extracellularly or intracellularly by various microorganisms such as bacteria, actinomycetes, and fungi, these enzymes exhibit diverse structures and serve a wide range of commercial applications. Of about 4,000 known microbial enzymes, around 200 types are used commercially, but only about 20 are produced on a truly industrial scale. These enzymes support approximately 150 industrial processes, including applications in pharmaceuticals, food production, and environmental management. The global industrial enzyme market is highly competitive.

And led by major players such as Novozymes, DSM, and DuPont. While North America and Europe are currently the largest consumers, the Asia-Pacific region, particularly China, Japan, and India, is witnessing rapid growth in enzyme demand due to expanding economies and industrial advancements. Let us look into the various microbial enzymes that are used in numerous industries. They are used in the field of microbiology and molecular biology, for example, DNA ligases, restriction enzymes, and polymerases, which are mostly DNA manipulative or DNA-modifying enzymes. Enzymes of microbial origin also have applications in the food and dairy industry.



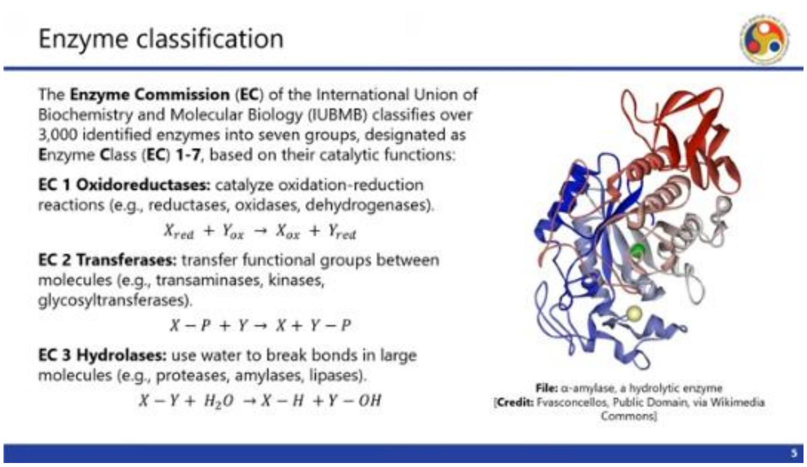
Examples include proteases, lipases, lactase, pectinase, amylase, amyloglucosidase, and phospholipase. They also have wide applications in the pharmaceutical and therapeutics fields, such as penicillin acylase and peroxidase. They are used in the paper and pulp industries, where amylase, lipase, protease, cellulase, hemicellulase, esterase, ligninase, and xylanase are applied. These enzymes are also used in the organic synthesis industry, such as lipases, oxidoreductases, glycosyltransferases, and lyases, as well as in the detergent industry, including amylase, cellulase, lipase, protease, and mannanase. They are also used in waste treatment.

Examples include amylases, cellulases, lipases, pectinases, proteases, laccase, and oxygenases. They are also used in the production of animal feed, such as phytase and xylanase. They are widely used in ethanol production, including cellulases, ligninases, and mannanase. They are used in the leather processing industry, such as proteases and lipases, and in the textile industry, including amylases, cellulases, pectinases, catalases, proteases, peroxidases, and keratinases. We see that some of these enzymes are common to many application fields, while others are specific to particular fields.

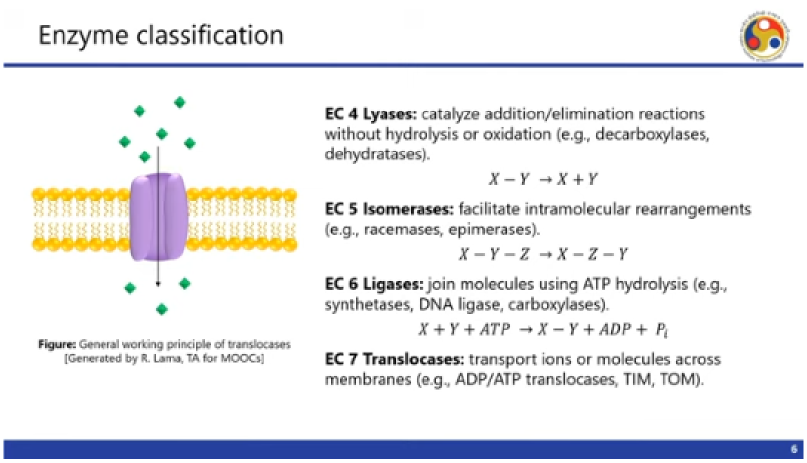


Now let us try to learn about the enzyme classification of the International Union of Biochemistry and Molecular Biology, shortly known as IUBMB, which classifies over 3000 identified enzymes into seven groups designated as enzyme classes EC1 to 7 based on their catalytic functions. For example, EC1, there are the oxidoreductases, these catalyze oxidation reduction reactions. Examples are reductases, oxidases and dehydrogenases. Then EC2 transferases, transfer functional groups between molecules. Example, transaminases, kinases and glycosyl transferases.

Then we have the EC3 hydrolysis, use water to break bones in large molecules, for example, proteases, amylases, and lipases. So then there is the EC4 lyases, this catalyzed addition elimination reactions without hydrolysis of oxidation. Examples are decarboxylases, dehydratases. Then we have the EC5 isomerases. This facilitates intramolecular rearrangements, example racemases and epimerases.



Then we have the EC6 ligases, the joint molecules using ATP hydrolysis, example synthesis, DNA ligases, carboxylases. Then we have EC7, the translocases, transport ions or molecules across membranes, example ADB, ATP translocases. In this figure, you can see the generic working principle of translocases. The enzyme nomoclaser system is a standardized method for naming and classifying enzymes established by the International Union of Biochemistry and Molecular Biology. This system classifies enzymes based on the type of reaction they catalyze and gives a unique identification number to each enzyme, ensuring uniformity and consistency in enzyme naming.



Each enzyme is assigned a unique numerical code, which consists of four parts, e.g. EC 2.7.1.1. This stands for hexokinase. The first digit designates the main class, specifying one of the seven main classes of enzymes. The second digit indicates the type of group or bond acted upon, or the subclass.

The third digit is the sub-subclass. It describes the reaction type or further categorizes the group affected. The fourth digit uniquely identifies the specific enzyme within the subclass. Now, let us have a comparative discussion between enzymatic synthesis and chemical synthesis. Chemical synthesis suffers from various disadvantages, such as requiring high temperature, low pH, and high pressure.

Enzyme nomenclature

- The enzyme nomenclature system is a standardized method for naming and classifying enzymes established by the International Union of Biochemistry and Molecular Biology (IUBMB).
- This system classifies enzymes based on the type of reaction they catalyze and gives a unique identification number to each enzyme, ensuring uniformity and consistency in enzyme naming.
- Each enzyme is assigned a unique numerical code, which consists of four parts (e.g., EC 2.7.1.1 for hexokinase):
  1. **First Digit (Main Class):** Specifies one of the seven main classes of enzymes.
  2. **Second Digit (Subclass):** Indicates the type of group or bond acted upon.
  3. **Third Digit (Sub-subclass):** Describes the reaction type or further categorizes the group affected.
  4. **Fourth Digit:** Uniquely identifies the specific enzyme within the sub-subclass.

It has low catalytic efficiency. It lacks enantiomeric specificity and uses organic solvents, which leads to organic waste and environmental pollution. On the other hand, enzymatic synthesis has many advantages. For example, unlike chemical synthesis, which requires high temperatures, enzymes can operate efficiently under moderate temperatures, neutral pH, and atmospheric pressure. Unlike the low catalytic efficiency of chemical synthesis, enzymatic synthesis offers better efficiency and has a long half-life.

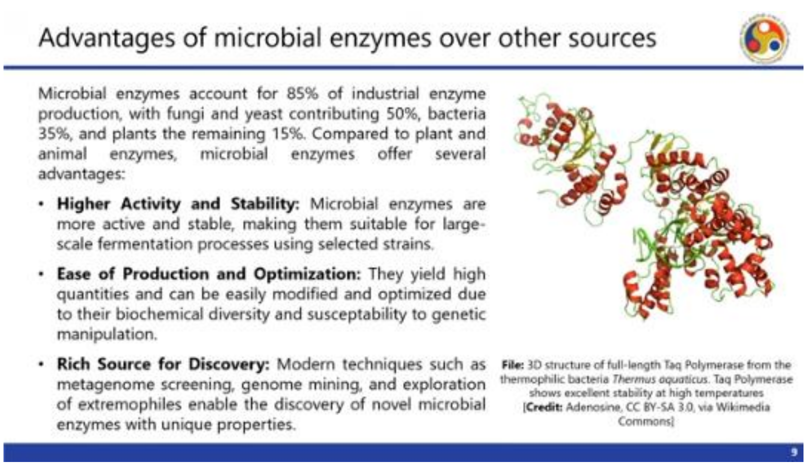
Similarly, the advantages of enzymatic synthesis provide high stereoselectivity, resulting in both stereochemically defined products and radiochemically defined reactions. Enzymatic synthesis, as opposed to the pollution caused by chemical synthesis, is eco-friendly. So, we see that today, in this age of climate change and sustainable development, enzymatic synthesis is going to play a very crucial role. So, what are the advantages of microbial enzymes over other sources? Microbial enzymes account for 85% of industrial enzyme production, with fungi and yeast contributing 50%, bacteria 35%, and plants the remaining 15%.

Enzymatic synthesis vs chemical synthesis

Disadvantages of chemical synthesis	Advantages of enzymatic synthesis
Requires high temperature, low pH, and high pressure	Can operate efficiently under moderate temperatures, neutral pH, atmospheric pressure
Low catalytic efficiency.	Better efficiency: long half-life
Lack of enantiomeric specificity	High stereo-selectivity, resulting in both stereo-chemically defined products and regio-chemically defined reactions.
Use of organic solvents leads to organic waste and environmental pollutants	Eco-friendly

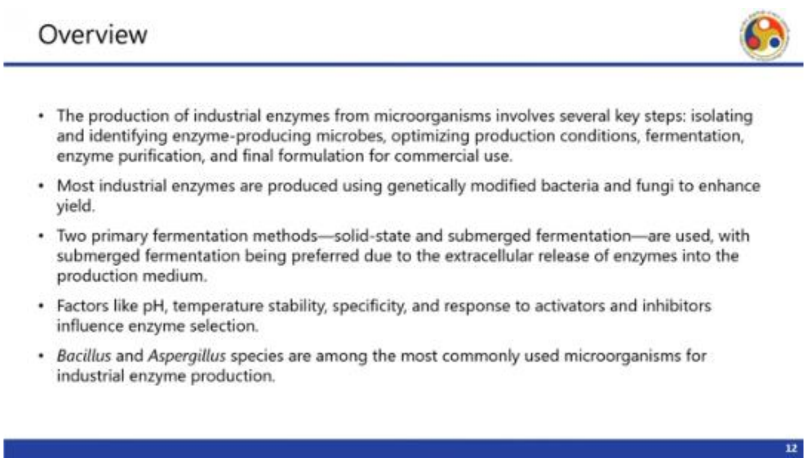
Compared to plant and animal enzymes, microbial enzymes offer several advantages, such as higher activity and stability. Microbial enzymes are more active and stable, making them suitable for large-scale fermentation processes using selected strains. It is also very easy to produce microbial enzymes and optimize the production process. They yield high quantities and can be easily modified and optimized due to their biochemical diversity and susceptibility to genetic manipulation. The microbial world offers rich sources of discovery.

Modern techniques such as metagenomic screening, genome mining, and exploration of extremophiles enable the discovery of novel microbial enzymes with unique properties. Now, let us move to the next section, where we will discuss the production of microbial enzymes. We start with a general outline of manufacturing enzymes. Then, we will discuss enzyme discovery through metagenomic screening and enzymes from extremophiles. We will also discuss enzyme inhibitors.



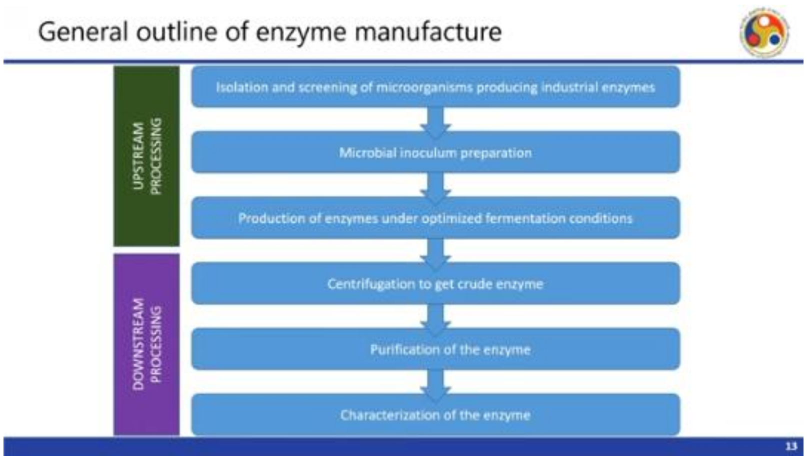
And fermentation conditions, the use of inexpensive substrates to cut down costs and optimization of the fermentation process. And once the enzyme is produced by the microbe, how do we purify those enzymes? The production of industrial enzymes from microorganisms involves several key steps, like isolating and identifying enzyme-producing microbes, optimizing production conditions, fermentation, enzyme purification, and final formulation for commercial use. Most industrial enzymes are produced using genetically modified bacteria and fungi to enhance yield. Two primary fermentation methods, solid-state and submerged fermentation, are used, with submerged fermentation being preferred due to the extracellular release of enzymes into the production medium.

Factors like pH, temperature stability, specificity, and response to activators and inhibitors influence enzyme selection. Bacillus and Aspergillus species are among the most commonly used microorganisms for industrial enzyme production. Let us discuss the general outline of enzyme manufacturing. So we have basically two important operations. One is the upstream set of operations, and the other is the downstream processing operations.

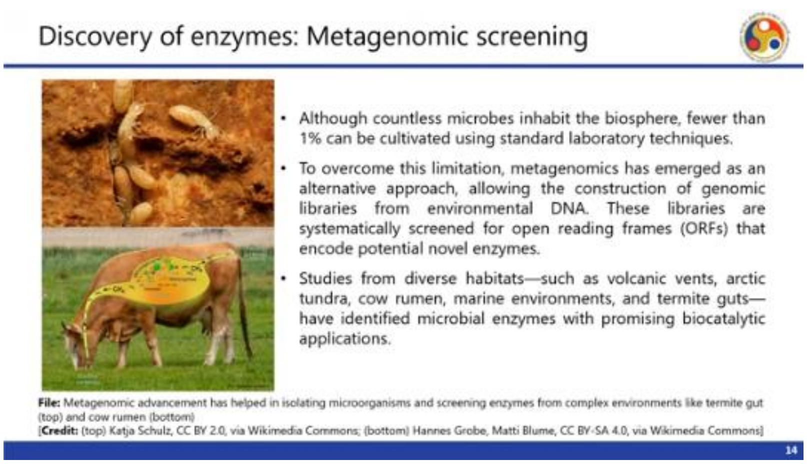


So in the upstream processing set of operations, we start with the isolation and screening of microorganisms, which will be producing these industrial enzymes. Then we go for the preparation of the microbial inoculum, then production of enzymes under optimized fermentation conditions. Once the enzyme is produced, we proceed to the downstream processing operations. It may be of different kinds.

We first centrifuge to get the crude enzyme and then also purify the enzyme further and then we also characterize the enzyme or go for quality control check. Now let us discuss a little bit about discovery of novel enzymes through the metagenomic screening approach. So, there are countless microbes in the biosphere, but only 1% of them can be cultivated. using the known laboratory techniques which we refer to as the standard laboratory techniques. To overcome this limitation, metagenomics has emerged as an alternative approach allowing the construction of genomic libraries from environmental DNA.

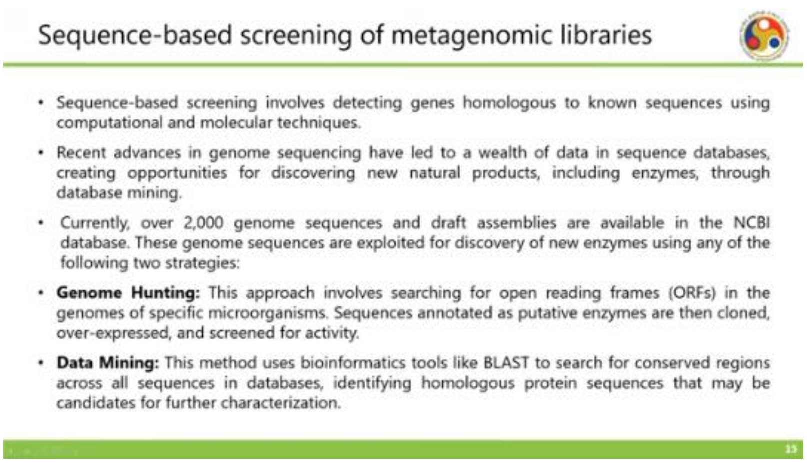


These libraries are systematically screened for open reading frames that encode potential novel enzymes. Studies from diverse habitats such as volcanic vents, Arctic tundra, cow rumen, marine environments and termite guts have identified microbial enzymes with promising biocatalytic applications. So, here you can see some termites and a cow and onto which a diagram of its rumen is superimposed. We use metagenomic advancements in isolating microorganisms and screening the enzymes from such complex environments. Then another approach is the sequence-based screening of metagenomic libraries.



Sequence-based screening involves detecting genes homologous to known sequences using computational and molecular techniques. Recent advances in genome sequences have led to a wealth of data in sequence databases, creating opportunities for discovering new natural products including enzymes through database mining. Currently, over 2000 genome sequences and draft assemblies are available in the NCBI database. These genome sequences are exploited for discovery of new enzymes using any of the following two strategies. The first strategy is the genome hunting.

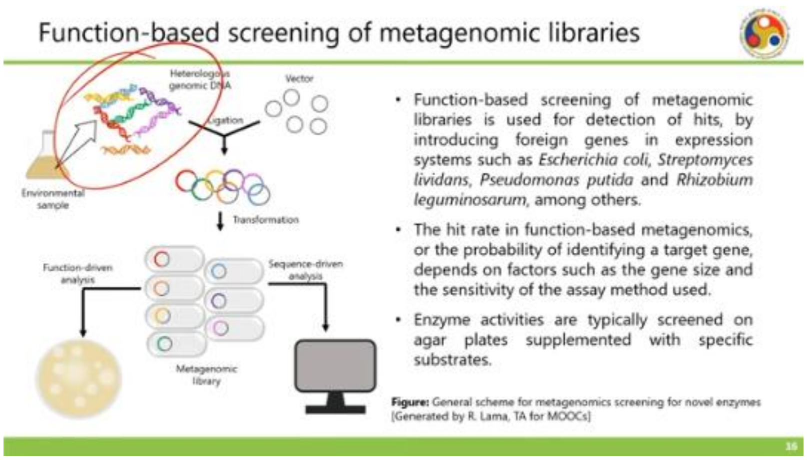
This approach involves searching for open reading frames or ORFs in a genome of specific microorganisms. Sequences annotated as putative enzymes are then cloned, overexpressed, and screened for activity. So, we do not clone the enzymes; we clone the gene sequences that produce these putative enzymes. So, the next step is data mining. This method uses bioinformatics tools like BLAST to search for conserved regions across all sequences in databases, identifying homologous protein sequences that may be candidates for further characterization.



Function-based screening of metagenomic libraries. So, function-based screening of metagenomic libraries is used for detecting hits by introducing foreign genes into expression systems such as Escherichia coli, Streptomyces lividans, Pseudomonas putida, and Rhizobium leguminosarum, among others. The hit rate in function-based metagenomics, or the probability of identifying a target gene, depends on factors such as the gene size and the sensitivity of the assay method used. So, here we have the environmental sample from which we obtain the heterologous genomic DNA, and then we clone it into certain vectors and transform them into host cells.

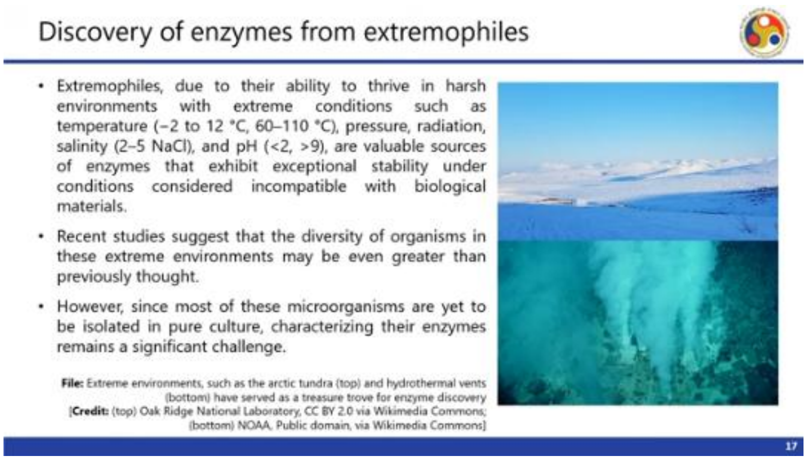
Then we proceed with two kinds of approaches. One is the function-driven analysis, and another is the sequence-driven analysis using these common metagenomic libraries. The enzyme activities are typically screened on agar plates supplemented with specific substrates. Now a question may arise: why do we need to go for cloning? So, I need to re-emphasize once again that only 1% of the known bacterial diversity species are culturable, while the remaining 99% are non-culturable.

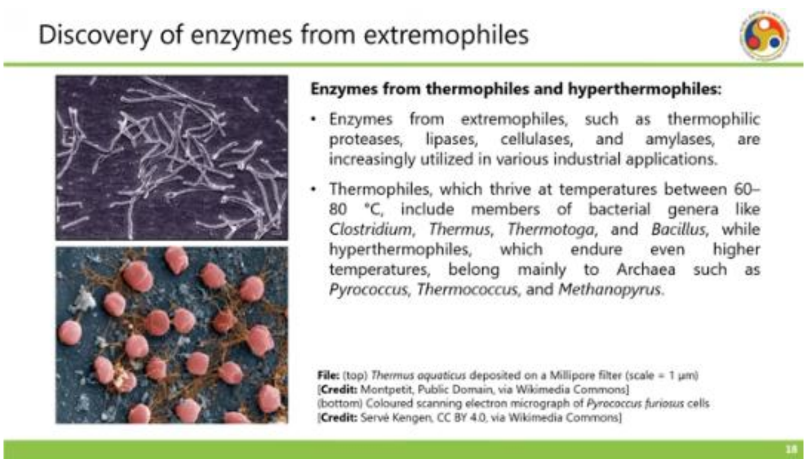
So, this DNA is basically targeted toward the non-culturable organisms. So, these are obtained, then cloned into vectors and transformed into bacterial strains that are culturable. So, by this method, we are able to generate a metagenomic library in a host bacterium that is culturable. So that the hidden useful genes in the non-culturable microbial population can be expressed, studied, or analyzed. Let us now discuss the discovery of enzymes from extremophiles.



As a term, extremophiles are already known to you. These are organisms that can survive in extreme conditions or thrive in harsh environments with extreme conditions such as temperatures ranging from very low to very high, from -2 to 12 degrees and then 60 to 110 degrees Celsius. They also endure high pressure, radiation, highly saline conditions like 2 to 5% NaCl, extremely low pH as low as 2, or high pH as high as 9. These extremophiles are valuable sources of enzymes that exhibit exceptional stability under conditions considered incompatible with biological materials. Recent studies suggest that the diversity of organisms in these extreme environments may be even greater than previously thought. However, since most of these microorganisms

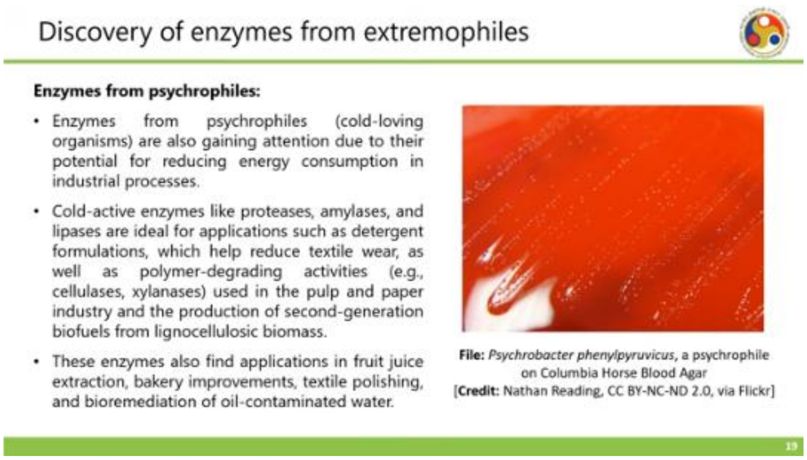
have yet to be isolated in pure culture, characterizing their enzymes remains a significant challenge. Here in this picture, you can see Thermus aquaticus deposited on a Millipore filter, and we know the importance of Thermus aquaticus, which provides us with Taq polymerase, about which we will discuss later. Enzymes from thermophiles and hyperthermophiles, such as proteases, lipases, cellulases, and amylases, are increasingly utilized in various industrial applications. Thermophiles, which can thrive at temperatures between 60 to 80 degrees Celsius or even up to 110 degrees Celsius, include members of bacterial genera like Clostridium, Thermus, Thermotoga, and Bacillus, while hyperthermophiles, which endure even higher temperatures, belong mainly to Archaea such as Pyrococcus, Thermococcus, and Methanopyrus.



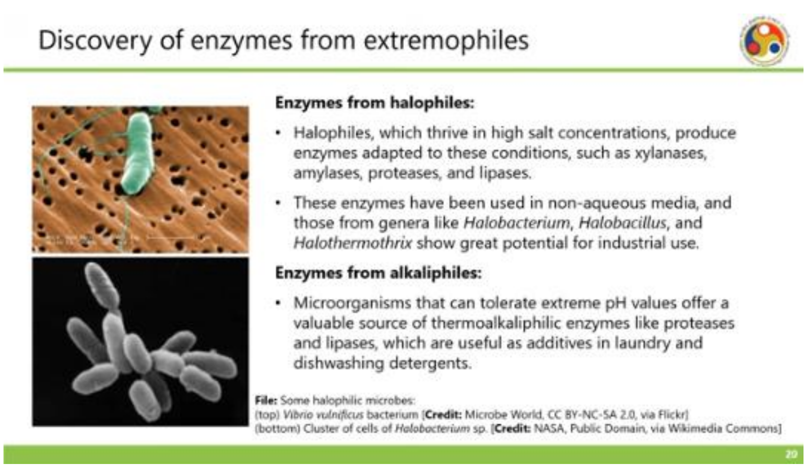


The Taq DNA polymerase from Thermus aquaticus alone generated $500 million in sales in 2009. Let us now discuss enzymes from psychrophiles. Enzymes from psychrophiles, or cold-loving organisms, are also gaining attention due to their potential for reducing energy consumption in industrial processes. In this picture is shown a Psychrobacter phenylpyruvicus, which is a psychrophile on Columbia horse blood agar. These collective enzymes, such as proteases, amylases, and lipases, are ideal for applications like detergent formulations, which help reduce textile wear, as well as polymer-degrading activities—for example, cellulases and xylanases used in the pulp and paper industry and the production of second-generation biofuels from lignocellulosic biomass.

These enzymes also find applications in fruit juice extraction, bakery improvements, textile polishing, and bioremediation of oil-contaminated water. Let us now discuss enzymes from halophiles. Halophiles thrive in high salt concentrations and produce enzymes adapted to these high-salt conditions. Some examples include xylanases, amylases, proteases, and lipases. These enzymes derived from halophiles have been used in non-aqueous media.

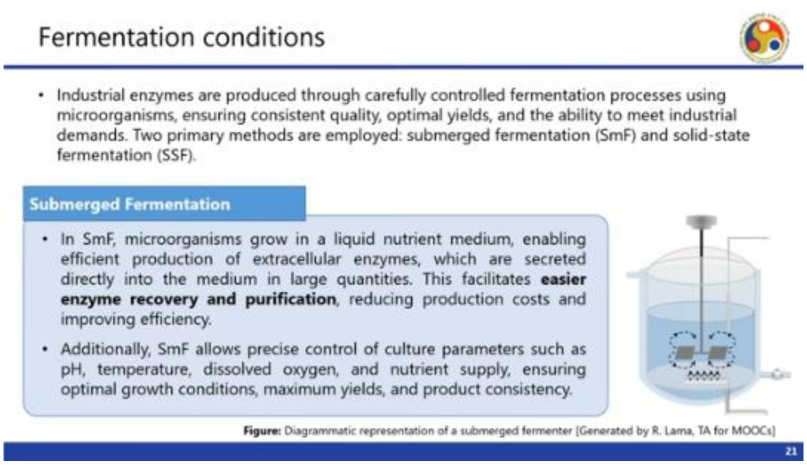


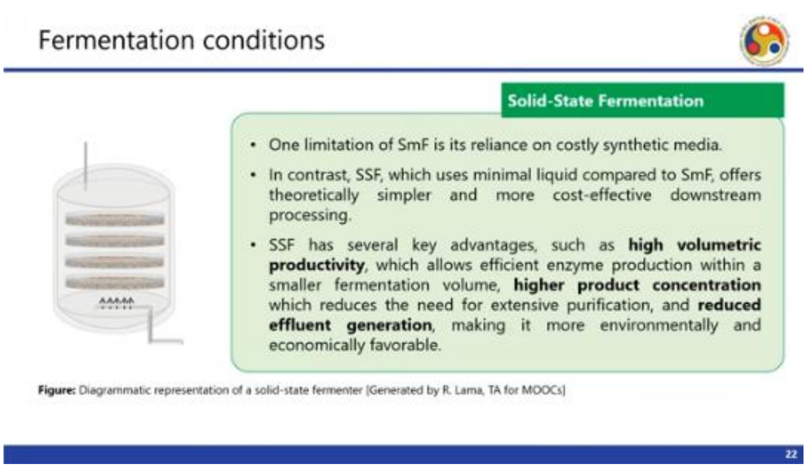
Those from genera like Halobacterium, Halobacillus, and Halothermotrix show great potential for industrial use. There are also enzymes that have been isolated from alkaliphiles. These are microorganisms that can tolerate extreme pH, offering a valuable source of thermoalkaliphilic enzymes like proteases and lipases, which are useful as additives in laundry and dishwashing detergents. Now, let us focus a little on the various fermentation conditions. Industrial enzymes are produced through carefully controlled fermentation processes using microorganisms, some of which were discussed earlier, ensuring consistent quality, optimal yields, and the ability to meet industrial demands.



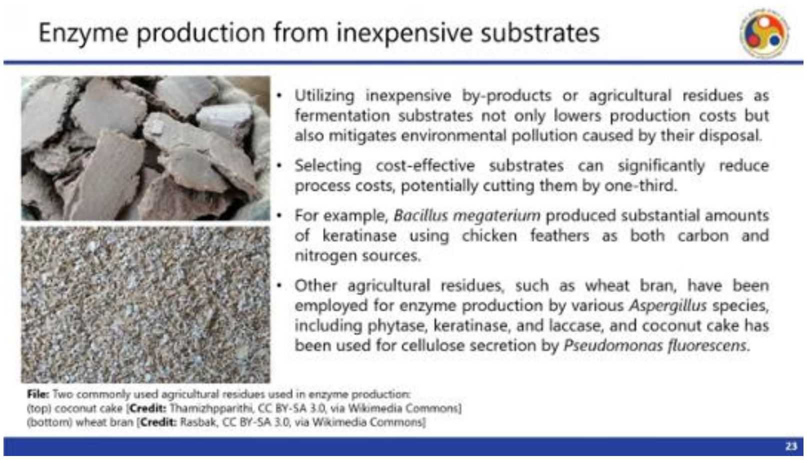
Two primary methods are employed: submerged fermentation and solid-state fermentation. Here in this picture, you can see the submerged fermentation. Here, microorganisms grow in a liquid nutrient medium, enabling efficient production of extracellular enzymes, which are secreted directly into the medium in large quantities. This facilitates easier enzyme recovery and purification, reducing production costs and improving efficiency.

Additionally, submerged fermentation allows precise control of culture parameters such as pH, temperature, dissolved oxygen, and nutrient supply, ensuring optimal growth conditions, maximum yields, and product consistency. Another type of fermentation is solid-state fermentation. The limitation of SMF is its reliance on costly synthetic media. In contrast, solid-state fermentation uses minimal liquid compared to SMF and offers a theoretically simpler and more cost-effective downstream processing. Solid-state fermentation has several key advantages, such as high volumetric productivity, which allows efficient enzyme production within a smaller fermentation volume; higher product concentration, which reduces the need for extensive purification; and reduced effluent generation, making it more environmentally and economically favorable.





Utilizing inexpensive bioproducts or agricultural residues as fermentation substrates not only lowers production costs but also mitigates environmental pollution caused by their disposal. Selecting cost-effective substrates can significantly reduce process costs, potentially cutting them by one-third. For example, Bacillus megatherium produced substantial amounts of keratinase using chicken feathers as both carbon and nitrogen sources. So here, we can see two commonly used agricultural residues for enzyme production: the coconut cake on the top and wheat bran below. Other agricultural residues have been employed for enzyme production by various Aspergillus species, including phytase, carotinase, and laccase, and coconut cake has been used for cellulose secretion by Pseudomonas fluorescens.



In this table, we can find many examples of enzymes produced from inexpensive substrates. Basically, the substrate is used to grow the microorganism that produces these enzymes. So, some of the substrates include, apart from chicken feathers, jamun leaves, molasses, and orange peel moistened with molasses. Then we have organic kitchen wastes, rice bran, rice bran, and wheat bran. Sawdust, sugarcane bagasse, sugarcane bagasse or straw, wheat bran, dry corn, sawdust, tea stalks, and wheat bran.

And then the microorganisms grown on these include, for example, in chicken feathers, we can grow Bacillus megatherium. Aspergillus species can be grown in jamun leaves, molasses, orange peel moistened with molasses, sugarcane bagasse, tea stalks, and wheat bran. Then we can grow Bacillus lehensis in rice bran and Penicillium on sawdust. And you can see the various enzymes produced by these organisms, which are listed in the central column. So, the production of industrial enzymes by microorganisms is heavily influenced by factors such as incubation time,

Table showing some enzymes produced from inexpensive substrates

Substrate used	Enzymes produced	Microorganism
Chicken feathers	Keratinase	*Bacillus megaterium*
Jamun leaves	Tannase	*Aspergillus* sp. GM4
Molasses	Neutral invertase	*Aspergillus* sp.
Orange peel moistened with molasses	Invertase	*Aspergillus sojae* JU12
Organic kitchen wastes	Amylase	*Chryseobacterium* sp., *Bacillus* sp.
Rice bran	Phytase	*Bacillus lehensis* MLB2
Rice bran and wheat bran	Laccase	*Stereum ostrea*
Saw-dust	Cellulase	*Penicillium* sp.
Sugarcane bagasse	Cellulase	*Aspergillus awamori*
Sugarcane bagasse or straw, wheat bran, dry corn, sawdust	Cellulase	*Pseudomonas fluorescens*
Tea stalks	Tannase	*Aspergillus tubingensis* CICC 2651
Wheat bran	Keratinase	*Aspergillus niger/flavus*
Wheat bran	Phytase	*Aspergillus niger*
Wheat bran	Xylanase	*Sphingobacterium* sp. SaH-05

Data adapted from Niyonzima, 2019 24

shaking agitation, initial pH, inoculum concentration, incubation temperature, carbon source, metal ions, and nitrogen sources. So, it is very important to optimize the fermentation conditions. Optimizing these factors is crucial for maximizing enzyme yields. Optimization involves fine-tuning media components, culture parameters, and fermentation conditions, This is typically done using a one-factor-at-a-time approach, where a single variable is optimized while keeping all others constant.

The optimized condition is then applied sequentially in subsequent experiments. This systematic optimization of nutritional, physicochemical, and fermentation parameters is essential for developing a cost-effective and efficient fermentation process, enabling the production of industrial enzymes in adequate quantities. Once the enzymes are produced by any of those fermentation methods we have discussed and grown on the various substrates we have discussed, we need to purify and isolate the enzymes. Commercial enzymes are produced in bioreactors and are initially obtained in crude form, requiring extraction and purification for further applications. These microbial enzymes are typically purified using three major approaches.

Optimization of fermentation conditions

- The production of industrial enzymes by microorganisms is heavily influenced by factors such as incubation time, shaking/agitation, initial pH, inoculum concentration, incubation temperature, carbon source, metal ions, and nitrogen source.
- Optimizing these factors is crucial for maximizing enzyme yields.
- Optimization involves fine-tuning media components, cultural parameters, and fermentation conditions.
- Typically, this is done using a one-factor-at-a-time approach, where a single variable is optimized while keeping all others constant. The optimized condition is then applied sequentially in subsequent experiments.
- This systematic optimization of nutritional, physico-chemical, and fermentation parameters is essential for developing a cost-effective and efficient fermentation process, enabling the production of industrial enzymes in adequate quantities.

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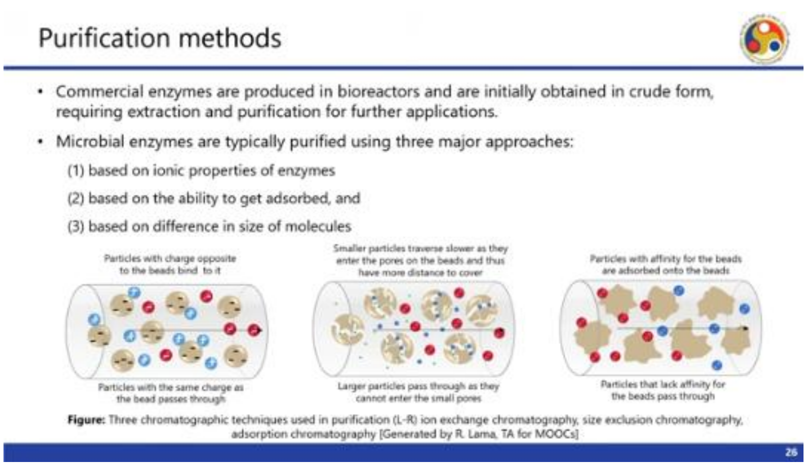
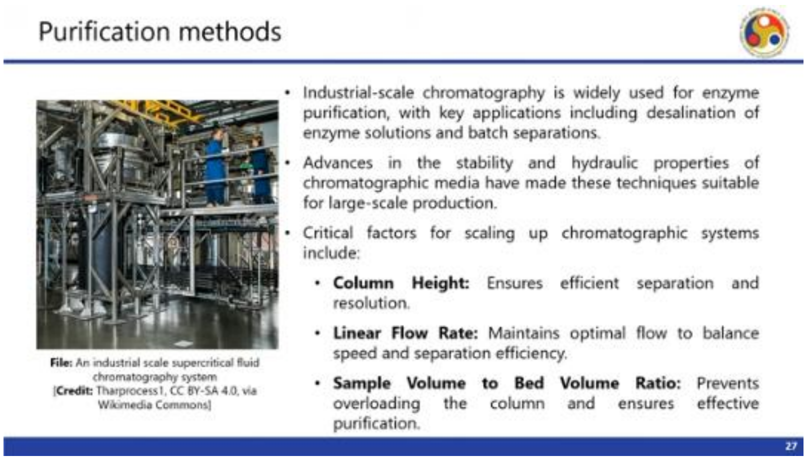
The first is based on the ionic properties of the enzyme. The second is based on the ability to be absorbed, and the third is based on differences in the size of molecules. In the first case, we can see ion-exchange chromatography, where particles with charges opposite to the bead bind to it, and this principle is used for purification. In the second case, we see size-exclusion chromatography, where smaller particles traverse slower as they enter the pores on the beads and thus have more distance to cover, whereas the larger particles, which do not enter these beads, will travel faster. Then we have adsorption chromatography, where particles with affinity for the beads are adsorbed onto the beads, while those without affinity move away quickly.

By exploiting these various properties based on different principles, we can purify the enzymes. Industrial-scale chromatography is widely used for enzyme purification, with key applications including desalination of enzyme solutions and batch preparations. Advances in the stability and hydraulic properties of chromatographic media have made these techniques suitable for large-scale production. Critical factors for scaling up chromatographic systems include the column height, which ensures efficient separation and resolution, and the linear flow rate, which maintains optimal flow to balance speed and separation efficiency. Then there is the sample volume to bed volume ratio, which prevents overloading the column and ensures effective purification.

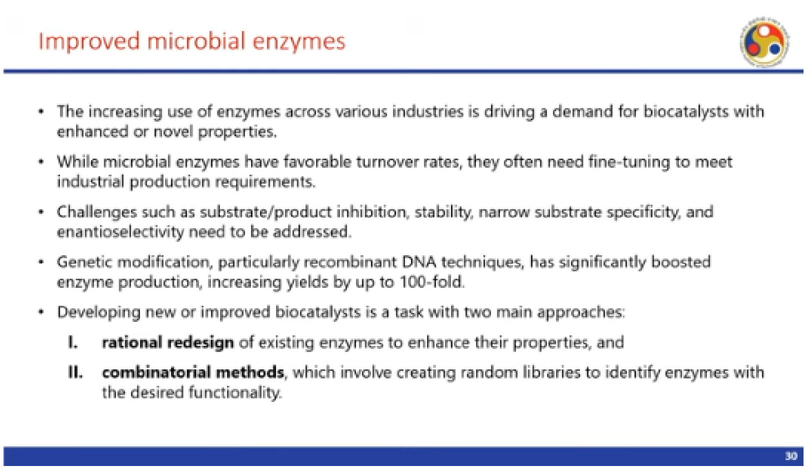


In this photo, you can see an industrial-scale supercritical fluid chromatographic system. We now move on to the next section, where we will discuss strategies to improve microbial enzymes. Here, we will discuss rational design, site-directed mutagenesis, and site-directed saturation mutagenesis. We will also discuss directed evolution, expression of recombinant enzymes, truncation, and fusion. The increasing use of enzymes across various industries is driving demand for biocatalysts with enhanced or novel properties.

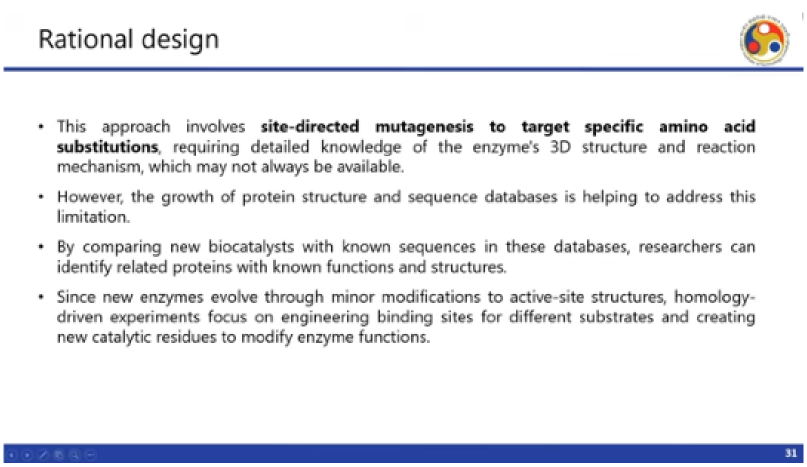


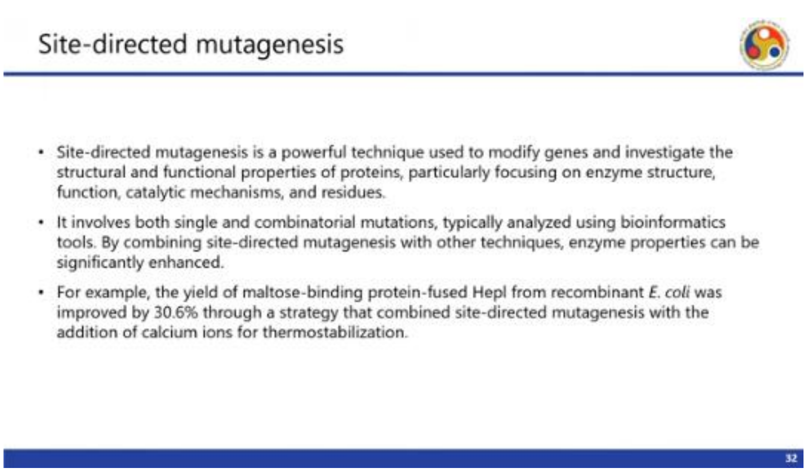
While microbial enzymes have favorable turnover rates, they often require fine-tuning to meet industrial production requirements. Challenges such as substrate or product inhibition, stability, narrow substrate specificity, and enantioselectivity need to be addressed. Genetic modification requires Particularly, recombinant DNA techniques have significantly boosted enzyme production, increasing yields up to 100-fold. Developing new or improved biocatalysts is a task with two main approaches.

The first one is the rational design of existing enzymes to enhance their properties. The second one is combinatorial methods, which involve creating random libraries to identify enzymes with the desired functionality. Let us start with rational design. This approach involves site-directed mutagenesis to target specific amino acid substitutions, requiring detailed knowledge of the enzyme's 3D structure and reaction mechanism, which may not always be available. However, the growth of protein structure and sequence databases is helping to address this limitation.



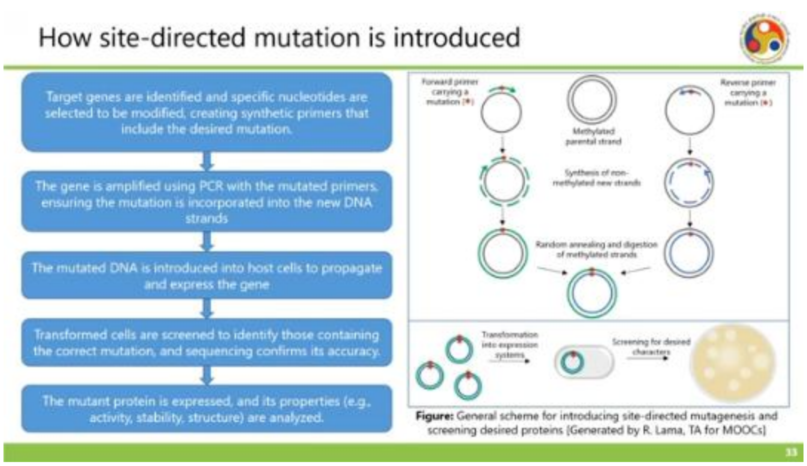
By comparing new bio-catalyst with known sequences in these databases, researchers can identify related proteins with known functions and structures. Since new enzymes evolve through minor modifications to active site structures, homology-driven experiments focus on engineering binding sites for different substrates and creating new catalytic residues to modify enzyme functions. Site directed mutagenesis is a powerful technique which is used to modify genes and investigate the structural and functional properties of proteins, particularly focusing on enzyme structure, function, catalytic mechanisms and residues. Side directed mutagenesis involves both single and combinatorial mutations and typically analyzed using bioinformatics tools by combining side directed mutagenesis with other techniques enzyme properties can be significantly enhanced. For example, the yield of maltose binding protein fused HEPI from recombinant E. coli was improved by 30.6% through a strategy that combined site-directed mutagenic acid with the addition of calcium ions for thermostabilization.



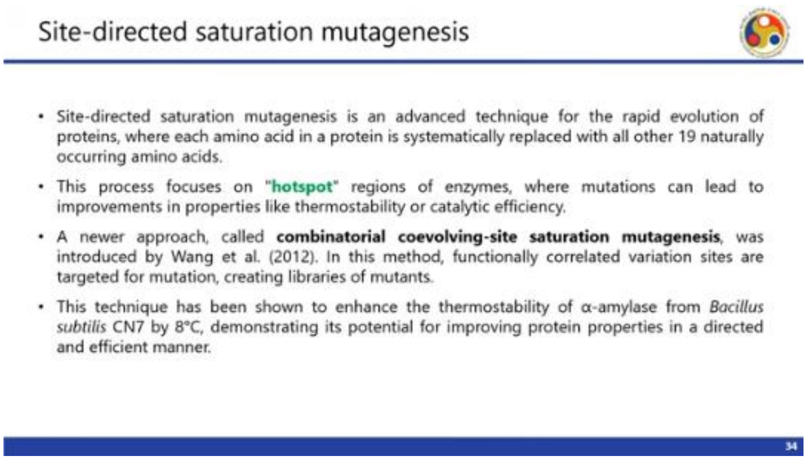


Let us now look how side directed mutagenesis is introduced. Target genes are identified and specific nucleotides are selected to be modified, creating synthetic primers that include the desired mutation. So, here we have a forward primer which is carrying the desired mutation or the changed nucleotide at this particular position as shown by these red asterisks. The gene is amplified using this particular primer by the PCR method, polymerase chain reaction method, ensuring the mutation is incorporated into the new DNA strains that is synthesized by this polymerase chain reaction. Then the mutated DNA is introduced into host cells to propagate and express the gene.

So, here we have parallelly also carried out the animal replication with a reverse primer which carries the complementary mutation and once both the strains are synthesized these are annealed and put together. So, these particular construct are now then used for transformation into the host. And then we finally go for screening of the desired mutants. So, transform cells are screened to identify those containing the correct mutation and we will go for DNA sequencing to confirm the accuracy. Then mutant proteins are expressed and its properties like activity, stability, structure are analyzed.



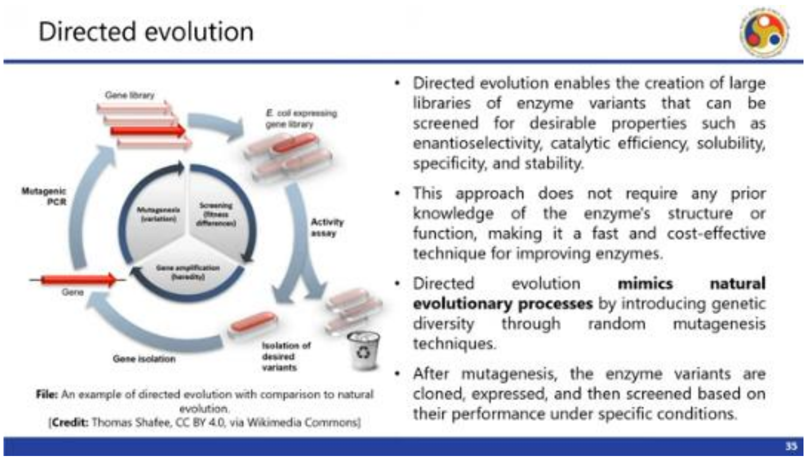
Site-directed saturation mutagenesis is an advanced technique for the rapid evolution of proteins, where each amino acid in a protein is systematically replaced with all other 19 naturally occurring amino acids. This process focuses on hotspot regions of enzymes where mutations can lead to improvements in properties like thermostability or catalytic efficiency. Another approach, called combinatorial co-evolving site saturation mutagenesis, was introduced by Wang et al. in 2012. In this method, functionally correlated variation sites are targeted for mutation, creating libraries of mutants. This technique has been shown to enhance the thermostability of alpha-amylase from Bacillus subtilis CN7 by 8 degrees Celsius, demonstrating its potential for improving protein properties in a directed and efficient manner.



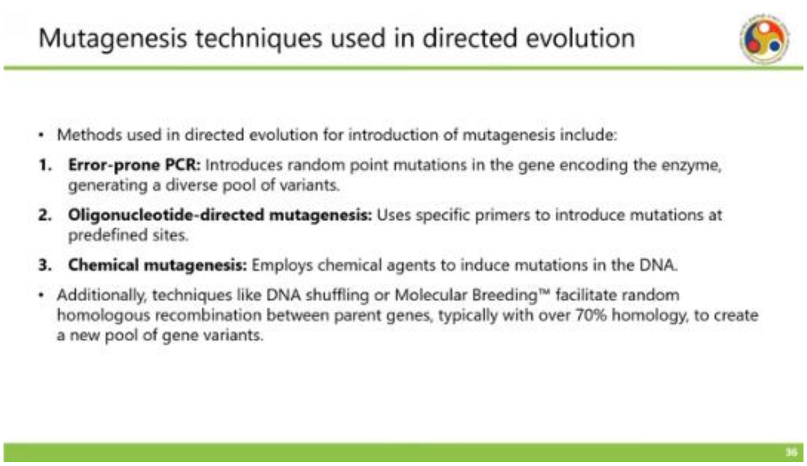
Let us now discuss directed evolution. This enables the creation of large libraries of enzyme variants that can be screened for desirable properties such as enantioselectivity, catalytic efficiency, solubility, specificity, and stability. This approach does not require any prior knowledge of the enzyme's structure or function, making it a fast and cost-effective technique for improving enzymes. Directed evolution mimics natural evolutionary processes by introducing genetic diversity through random mutagenesis techniques. After mutagenesis, the enzyme variants are cloned, expressed, and then screened based on their performance under specific conditions.

So, this is one of the most important techniques. Here, we have a gene, for example, where we perform mutagenic PCR and create a gene library or pool. These are all cloned and expressed in a host like E. coli, and then we perform an activity assay. The clones which do not have any activity or the desired activity are rejected, and the clones which have the desired variants are isolated and then can be moved into the next cycle. So, with every cycle, there is a process of evolution taking place, but this evolution is not random; it is directed evolution because we are using mutagenic PCR to influence this evolutionary process.

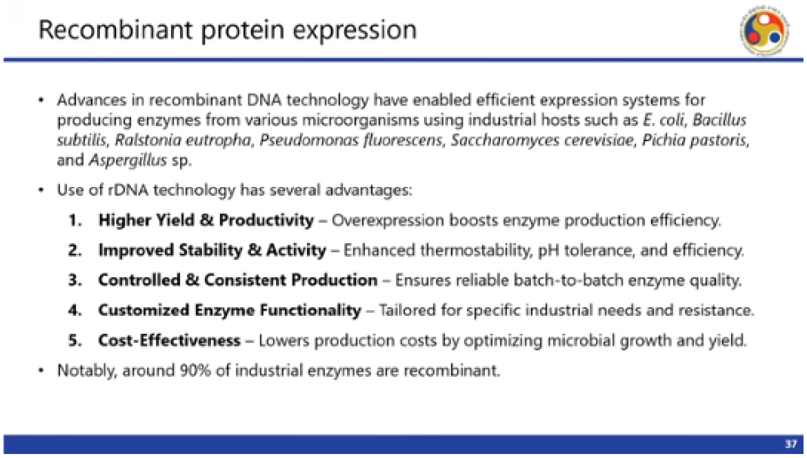
This mimics the natural evolutionary process by introducing genetic diversity through random mutagenesis techniques, selecting the desired variants at every cycle, and subjecting them to further evolutionary pressure. So, the mutagenesis techniques used in directed evolution to introduce variation include error-prone PCR. This introduces random point mutations in the gene encoding the enzyme, generating a diverse pool of variants. Then, oligonucleotide-directed mutagenesis. This uses specific primers to introduce mutations at predefined sites.



Then, we may also use chemical mutagenesis, where chemical agents are employed to introduce mutations in the DNA. And then, there are several other techniques like DNA shuffling or molecular breeding. These facilitate random homologous recombination between parent genes, typically with over 70% homology, to create a new pool of gene variants. Let us now discuss recombinant protein expression. Advances in recombinant DNA technology have enabled efficient expression systems for producing enzymes from various microorganisms using industrial hosts such as E. coli, Bacillus subtilis, Ralstonia eutropha, Pseudomonas fluorescens, Saccharomyces cerevisiae, Pichia pastoris, and Aspergillus species.



The use of recombinant DNA technology has several advantages. Number one, higher yield and productivity. Overexpression boosts enzyme production efficiency, improves stability and activity, enhances thermal stability, pH tolerance, and efficiency. Control and consistent production ensure reliable batch-to-batch enzyme quality, customized enzyme functionality tailored for specific industrial needs, and resistance. Cost-effectiveness lowers production costs by optimizing microbial growth and yields. Notably, around 90% of industrial enzymes are recombinant.



What are the steps in recombinant DNA technology? This is also discussed in other sections. Let us just have a quick overview. Here is a plasmid vector. Plasmids are extra-chromosomal DNA, as you all know, which help the host overcome stress conditions.

But we also use these plasmids for transferring foreign genes. From another source, we isolate the gene of interest, then perform restriction endonuclease digestion of both the plasmid vector and the gene cut from the genomic DNA, and ligate them. This construct is then used for transforming the host, and we screen the cells that are thereby transformed. This is the general overall scheme. So, isolation of the vector gene, isolation of the target gene, cleavage of isolated DNA at specific sites by restriction enzymes, ligation of the target gene with the vector DNA, introduction of rDNA into compatible host cells, replication and expression of rDNA within the host cells, and screening of the transformed cells.

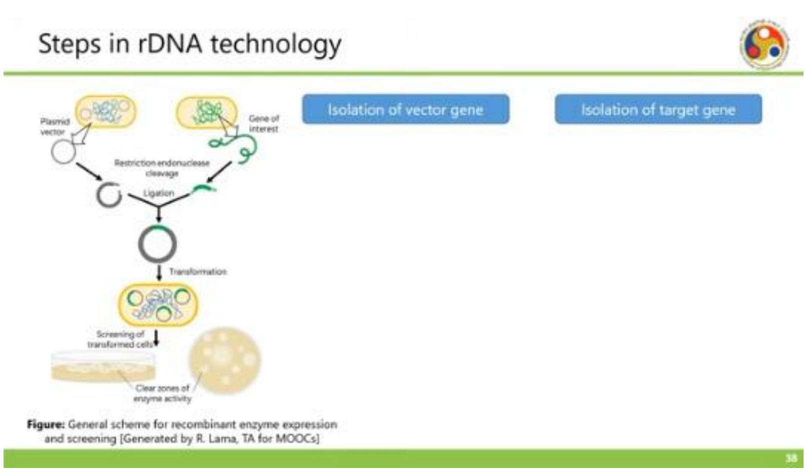
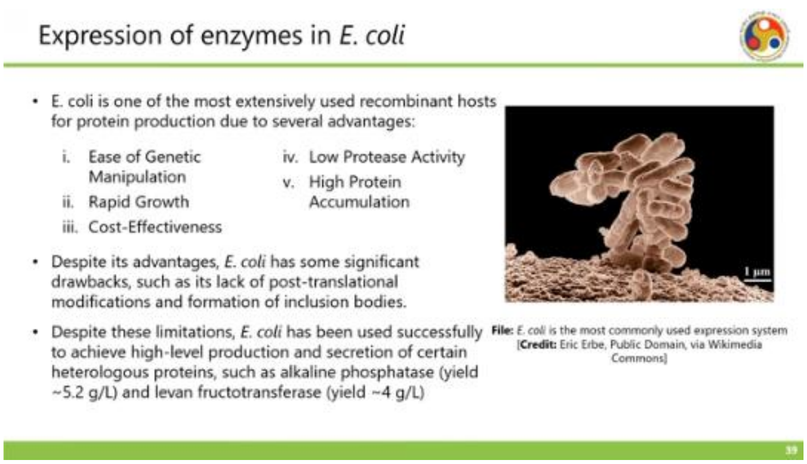


Figure: General scheme for recombinant enzyme expression and screening [Generated by R. Lama, TA for MOOCs]

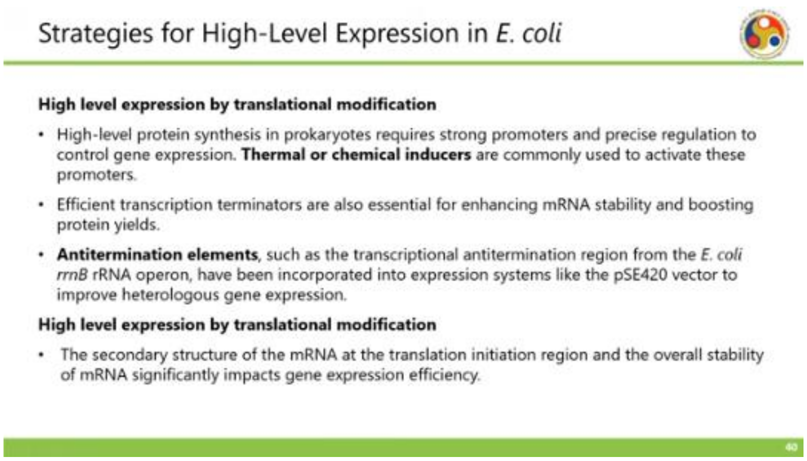
Expression of enzymes in E. coli by this cloning method. E. coli is one of the most extensively used recombinant hosts for protein production due to several advantages. First, it is very easy to manipulate genetically. It grows very fast and is cost-effective when it comes to its culture and maintenance. It also has very low protease activity and high protein accumulation.

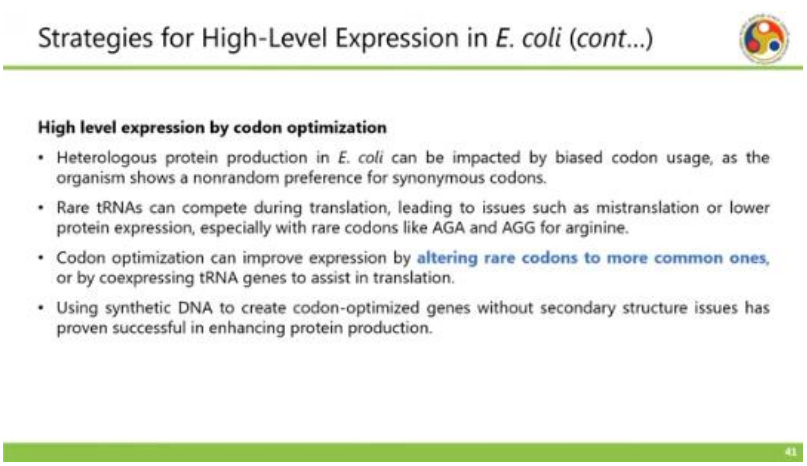
The low protease activity helps us in obtaining more proteins. Otherwise, if the protease activity is high, that will actually degrade the proteins that are produced by this process. However, despite its advantages, E. coli has some significant drawbacks, such as its lack of post-translational modification and formation of inclusion bodies. Despite these limitations, E. coli has been used successfully to achieve high-level production and secretion of certain heterologous proteins such as alkaline phosphatases and levan fructotransferases. Let us discuss about the strategies of high-level expression in E. coli.



High-level expression by translational modification. High-level protein synthesis in prokaryotes requires strong promoters and precise regulation to control gene expression. Thermal or chemical inducers are commonly used to activate these promoters. Efficient transcription terminators are also essential for enhancing mRNA stability and boosting protein yields. Anti-termination elements such as the transcriptional anti-termination regions from the E. coli, rrNB, rRNA operon have been incorporated into the expression systems like the pSE420 vector to improve heterologous gene expression.

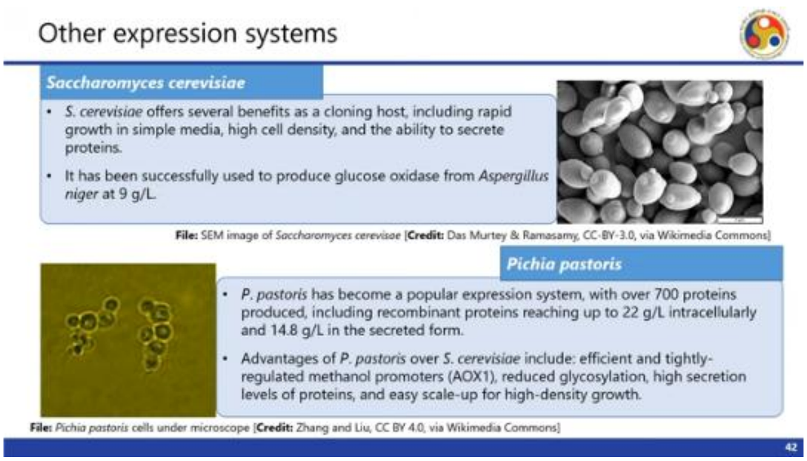
High level expression by translational modification, the secondary structure of the mRNA at the translation initiation region and the overall stability of mRNA significantly impacts gene expression efficiency. Then we also can increase the level of expression by codon optimization. Heterologous protein production in E. coli can be impacted by biased codon usage as the organism shows a non-random reference for synonymous codons. Rare tRNAs can compete during translation leading to issues such as mistranslation or lower protein expression, especially with rare codons like AGA and AGG for arginine. Codon optimization can improve expression by altering rare codons to more common ones or by co-expressing tRNA genes to exist in translation using synthetic DNA to create codon optimization genes without secondary structure issues has proven successful in enhancing protein production.



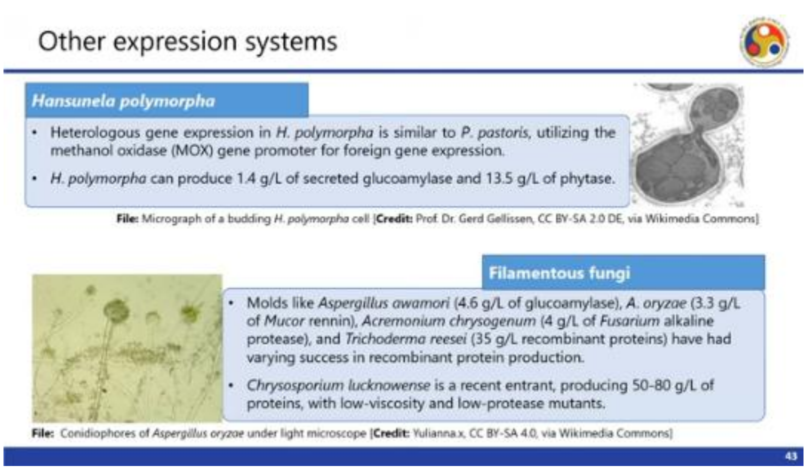


So, some of the other expression systems, other than E. coli, include Saccharomyces cerevisiae. Saccharomyces cerevisiae offers several benefits as a cloning host, which include rapid growth in simple media, high cell density, and the ability to secrete proteins. It has been successfully used to produce glucose oxidase from Aspergillus niger at 9 grams per liter. Then we have Pichia pastoris, which has become a popular expression system with over 700 proteins produced, including recombinant proteins, reaching up to 22 grams per liter compared to 9 grams per liter in Saccharomyces cerevisiae intracellularly and 14.8 grams per liter in secreted form, which is again higher than that produced by Aspergillus niger at 9.2 grams per liter.

These advantages of Pichia pastoris over Saccharomyces cerevisiae therefore include efficient and tightly regulated methanol promoters, reduced glycosylation, high secretion levels of proteins, and easy scale-up for high-density growth. Then we have another expression system, Hansenula polymorpha. Heterologous gene expression in Hansenula polymorpha is similar to Pichia pastoris, utilizing the methanol oxidase gene promoter for foreign gene expression. It can produce around 1.4 grams per liter of secreted glucoamylase and 13.5 grams per liter of phytase. Filamentous fungi are also used as an expression system.

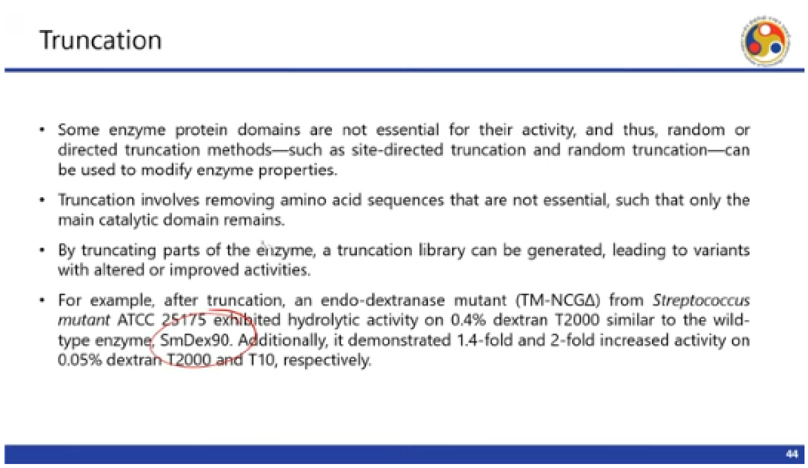


For example, molds like Aspergillus awamori produce around 4.6 grams per liter of glucoamylases. Then Aspergillus oryzae, 3.3 grams per liter of Mucor rennin. Acremonium chrysogenum, 4 grams per liter of Fusarium alkaline protease, and Trichoderma reesei, 35 grams per liter of recombinant proteins, which is quite impressive. These have had various successes in recombinant protein production. Chrysosporium lucknowense is a recent entrant, producing around 50 to 80 grams per liter of proteins with low viscosity and low protease mutants.



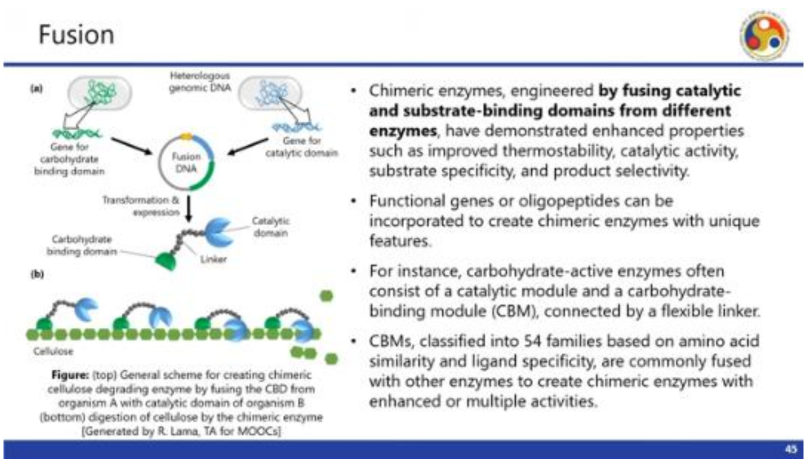
Some enzyme protein domains are not essential for their activity. Thus, random or directed truncation methods to remove them, such as site-directed truncation and random truncation, can be used to modify these enzyme properties. So, truncation is an important step by which we can enhance the properties of certain enzymes. This involves removing amino acid sequences that are not essential, such that only the main catalytic domain remains. By truncating parts of the enzyme, a truncation library can be generated, leading to variants with altered or improved activities.

For example, after truncation, an endo-dextranase mutant (TM-NCZ-delta), where 'delta' stands for the truncation or deletion from Streptococcus mutant ATCC 25175, exhibited hydrolytic activity on 0.4% dextran T2000 similar to the wild-type enzyme SM-DEX90. Additionally, it demonstrated 1.4-fold and 2-fold increased activity on 0.05% dextran T2000 and T10, respectively. Another technique is that of creating fusion proteins, which are also called chimeric proteins or chimeric enzymes. These are engineered by fusing catalytic and substrate-binding domains from different enzymes. They have demonstrated enhanced properties, such as improved thermostability, catalytic activity, substrate specificity, and product selectivity.



Here, in this illustration (Figure A), we can see a general scheme for creating a chimeric cellulose-degrading enzyme by fusing the carbohydrate-binding domain from organism A with the catalytic domain of organism B (below) for cellulose digestion by the chimeric enzyme. Here, this is the gene for the carbohydrate-binding domain, and here is the gene for the catalytic domain. These are fused to create fusion DNA, which is then transformed and expressed. Now, you have a fusion protein where the carbohydrate-binding domain from one source and the catalytic domain from another source are linked by a linker in between. In Figure B, this fusion protein or chimeric enzyme is used for degrading cellulose.

Functional genes or oligopeptides can be incorporated to create chimeric enzymes with unique features. So here, we have already discussed the catalytic module and the carbohydrate-binding module connected by this flexible linker. CBMs, classified into 54 families based on amino acid similarity and ligand specificity, are commonly fused with other enzymes to create chimeric enzymes with enhanced or multiple activities. Let us now discuss the applications of microbial enzymes in various industries, particularly their use in organic synthesis industries, therapeutics or pharmaceuticals, diagnostics, food and beverage industries, animal feed production, paper and pulp industries, textile industries, leather industries, detergent industries, and waste management.



So, this table shows some of the applications of enzymes in various industries. For example, in food and beverage industries, we use proteases, lipases, lactases, pectinases, amylases, lecases, amyloglucosidases, phospholipases for the degradation of starch and proteins into sugars, production of low-calorie beer, fruit juice processing, cheese production, glucose production from lactose, dough stability, and conditioning. Similarly, in the detergent industry, we use amylase, cellulase, lipase, protease, mannanase for removing protein stains, cleaning agents, removing insoluble starch, fats, and oils to increase the effectiveness of detergents. In the textile industry, we use amylase, cellulase, pectinase, catalase, protease, peroxidase, keratinase for fabric finishing.

In denims, wool treatment, degumming of raw silk, or biopolishing, cotton softening. And in the animal feed industry, we use phytase and xylanase for increasing total phosphorus content for growth and digestibility. In the ethanol production industry, we use cellulases, ligninase, mannanase for the formation of ethanol. In the paper and pulp industry, we use amylase, lipase, protease, cellulase, hemicellulose, esterase, ligninase, xylanase for degrading starch to lower viscosity, aiding sizing, deinking, and coating paper; cellulose and hemicellulose smooth fibers, enhance water drainage, and promote ink removal. Lipases reduce... Peach and lignin is removed to soften paper.

Table showing some applications of enzymes in various industries

Industry	Name of enzymes	Applications
Food, dairy and beverage	Protease, lipase, lactase, pectinase, amylase, laccase, amyloglucosidase, phospholipase	Degradation of starch and proteins into sugars, Production of low caloric beer, Fruit juice processing, Cheese production, Glucose production from lactose, Dough stability and Conditioning.
Detergents	Amylase, cellulase, lipase, protease, mannanase	Remove protein after staining, Cleaning agents, Removing insoluble starch, fats and oils, To increase effectiveness of detergents.
Textiles	Amylase, cellulase, pectinase, catalase, protease, peroxidase, keratinase	Fabric finishing in denims, Wool treatment, Degumming of raw silk (biopolishing), Cotton softening.
Animal feed	Phytase, xylanase	Increase total phosphorus content for growth, Digestibility

Data adapted from Liu & Kokare, 2017 48

Then, in the later industry, we use proteases and lipases, unhairing, batting, and depaking. And in the pharmaceuticals, we use penicillin acylase, peroxidase for the synthesis of semi-synthetic antibiotics and production of antimicrobials. In molecular biology, we use DNA ligase, restriction enzymes, and polymerases to manipulate DNA in genetic engineering, DNA restriction, polymerase chain reaction, and also in forensic sciences. Now, let us discuss the application of microbial enzymes in organic synthesis. Enzyme-based processes are increasingly important.

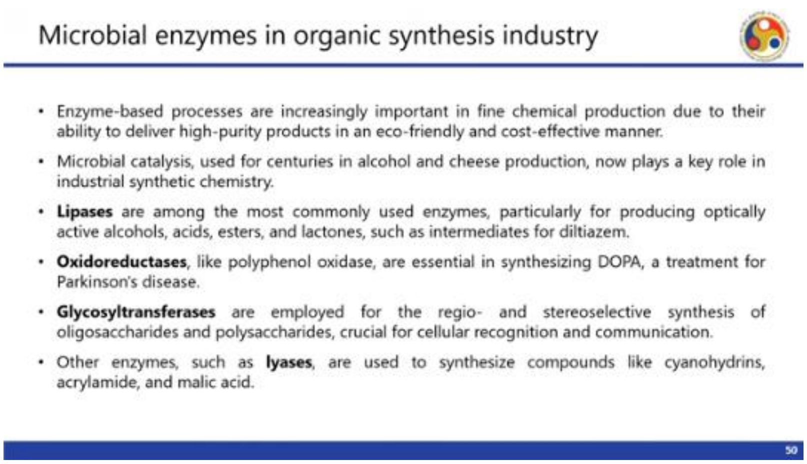
Table showing some applications of enzymes in various industries

Industry	Name of enzymes	Applications
Ethanol production	Cellulase, ligninase, mannanase	Formation of ethanol
Paper and pulp	Amylase, lipase, protease, cellulase, hemicellulase, esterase, ligninase, xylanase	Degrade starch to lower viscosity, aiding sizing, deinking, and coating paper. Cellulase and hemicellulase smooth fibers, enhance water drainage, and promote ink removal. Lipases reduce pitch and ligninase remove lignin to soften paper.
Leather	Protease, lipase	Unhearing, bating, depicking
Pharmaceuticals	Penicillin acylase, peroxidase	Synthesis of semisynthetic antibiotics, Antimicrobials
Molecular biology	DNA ligase, restriction enzymes, polymerase	Manipulate DNA in genetic engineering. DNA restriction and the polymerase chain reaction. Important in forensic science.

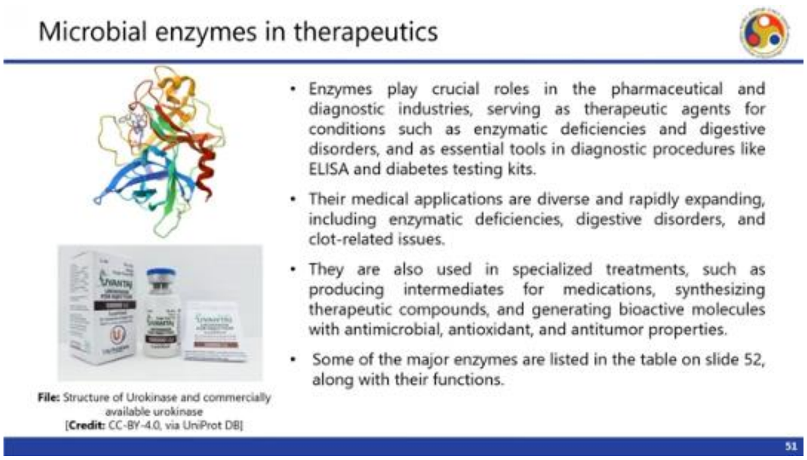
Data adapted from Liu & Kokare, 2017 49

In fine chemical production due to their ability to deliver high-purity products in an eco-friendly and cost-effective manner. Microbial catalysts, used for centuries in alcohol and cheese production, now play a key role in industrial synthetic chemistry. Lipases are among the most commonly used enzymes, particularly for producing optically active alcohols, acids, esters, and lactones, such as intermediates for diltiazem. Oxidoreductases like polyphenol oxidase are essential in synthesizing DOPA. A treatment for Parkinson's disease.

Then, the glycosyl transferases, which are employed for the regio- and stereoselective synthesis of oligosaccharides and polysaccharides, are crucial for cellular recognition and communication. Other enzymes, such as lyases, are used to synthesize compounds like cyanohydrins, acrylamide, and malic acid. Now, let us discuss the use of microbial enzymes in pharmaceuticals or therapeutics. Enzymes play crucial roles in the pharmaceutical and diagnostic industries, serving as therapeutic agents for conditions such as enzymatic deficiencies and digestive disorders, and are essential tools in diagnostic procedures like ELISA and diabetes testing kits. We will discuss diagnostics separately.



So, the medical applications of microbial enzymes are diverse and rapidly expanding including enzymatic deficiencies, digestive disorders and clot related issues. They are also used in specialized treatments such as producing intermediates for medications, synthesizing therapeutic compounds and generating bioactive molecules with antibacterial antioxidants and anti-tumor properties. Some of the major enzymes are listed in the table on slide 52 along with their functions. So, here we have enzymes like L-asparagin, L-glutaminase, L-tyrosinase, galactosidase which are used for treating tumours and the microorganisms which are used for producing these are the E. coli, Pseudomonas acidovorans, Beauveria bassiana and Acinetobacter. Then superoxidase, dismutase and serrapeptase are anti-inflammatory agents produced in lactobacillus, nocardia and microbacterium.



Streptokinase, urokinase acts as anticoagulants produced in streptococci, bacillus. And then penicillin oxidase, rifamycin, B-oxidase, these are used for antibiotic synthesis produced in penicillin. And superoxide dismutase, glutathione peroxidase catalyst acts as antioxidants and these are produced in lactobacillus plantarum and C glutamicum. Then we have collagenase which are used for treating skin ulcers produced in clostridium perfringens. Then we have lacnes, rhodanese, they are used as detoxification agents and they are produced in pseudomonas aeruginosa.

Then we have beta-lactamase, which are associated with antibiotic resistance, produced in Klebsiella pneumoniae and Cytobacter freundi or Serratia marcescens. Then we have the uricase, which are treatment for gout produced in aspergillus flavus. Similarly, we have alpha amylase and lipase used in digestive disorders produced in bacillus species, etc. And rhodanase, which is used in cyanide poisoning treatment and produced in sulfobacillus sibiricus. Let us now discuss about the application of microbial enzymes in the diagnostic area.

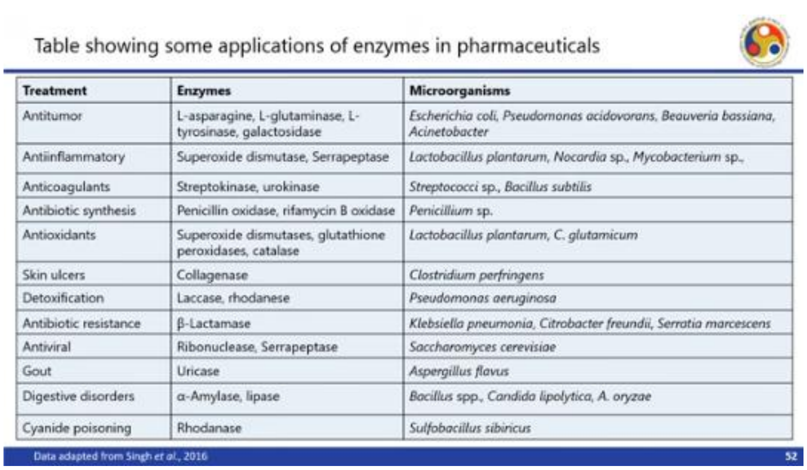


Table showing some applications of enzymes in pharmaceuticals

Treatment	Enzymes	Microorganisms
Antitumor	L-asparagine, L-glutaminase, L-tyrosinase, galactosidase	*Escherichia coli, Pseudomonas acidovorans, Beauveria bassiana, Acinetobacter*
Antiinflammatory	Superoxide dismutase, Serrapeptase	*Lactobacillus plantarum, Nocardia* sp., *Mycobacterium* sp.,
Anticoagulants	Streptokinase, urokinase	*Streptococci* sp., *Bacillus subtilis*
Antibiotic synthesis	Penicillin oxidase, rifamycin B oxidase	*Penicillium* sp.
Antioxidants	Superoxide dismutases, glutathione peroxidases, catalase	*Lactobacillus plantarum, C. glutamicum*
Skin ulcers	Collagenase	*Clostridium perfringens*
Detoxification	Laccase, rhodanese	*Pseudomonas aeruginosa*
Antibiotic resistance	β-Lactamase	*Klebsiella pneumonia, Citrobacter freundii, Serratia marcescens*
Antiviral	Ribonuclease, Serrapeptase	*Saccharomyces cerevisiae*
Gout	Uricase	*Aspergillus flavus*
Digestive disorders	α-Amylase, lipase	*Bacillus* spp., *Candida lipolytica, A. oryzae*
Cyanide poisoning	Rhodanase	*Sulfobacillus sibiricus*

Data adapted from Singh *et al.*, 2016

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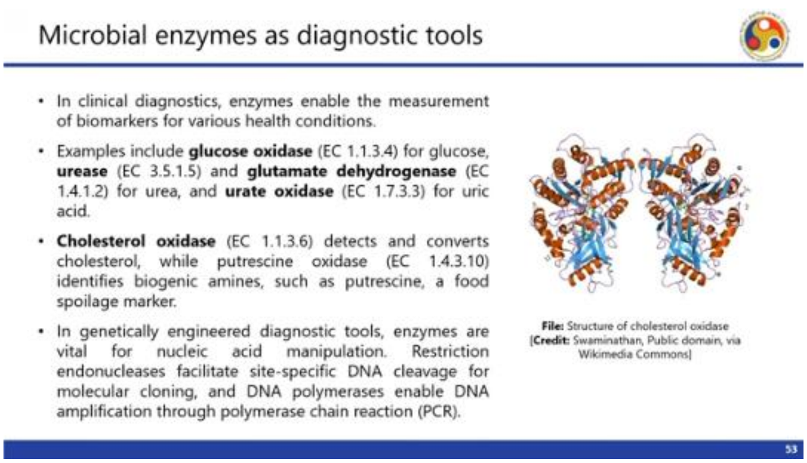
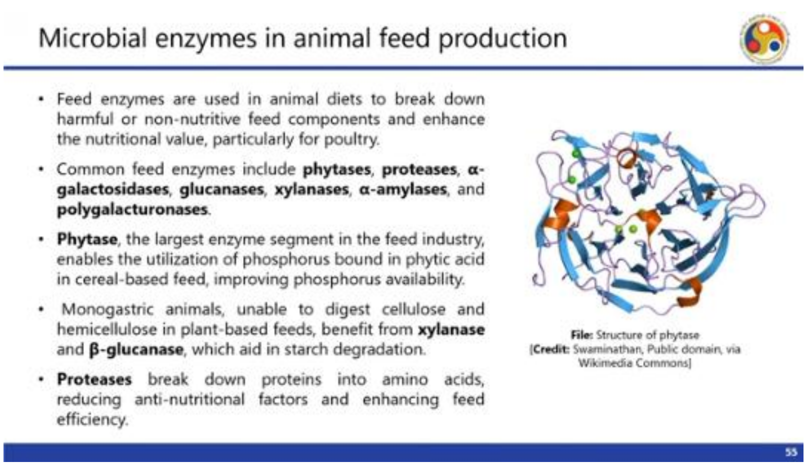
These enzymes enable the measurement of biomarkers for various health conditions. Some examples include glucose oxidase for glucose, urease and glutamate dehydrogenase for urea, and urate oxidase for uric acid. Cholesterol oxidase detects and converts cholesterol, while putrescine oxidase identifies biogenic amines such as putrescine, a food spoilage marker. In genetically engineered diagnostic tools, enzymes are vital for nucleic acid manipulation. Restriction endonucleases facilitate site-specific DNA cleavage for molecular cloning, and DNA polymerase enables DNA amplification through polymerase chain reaction. Next comes the application of microbial enzymes in the food and beverage industry.



Understanding the roles of enzymes in food manufacturing and the ingredients industry has enhanced processes, leading to safer, higher-quality products. Enzymes have expanded into new areas like fat modification and sweetener technology. Enzymes in this industry are used to improve brewing consistency, enhance the nutritional properties of proteins, and increase juice yield while improving color and aroma. The applications span baking, dairy, juice production, and brewing. In baking, microbial enzymes improve dough stability, crumb texture, and product shelf life.

In dairy, enzymes are essential for cheese processing, making it the second-largest application area, followed by beverages. Microbial enzymes are also used in the production of animal feed. Feed enzymes are used in animal diets to break down harmful or non-nutritive feed components and enhance nutritional value, particularly for poultry. Common feed enzymes include phytases, proteases, alpha-galactosidases, glucanases, xylanases, alpha-amylases, and polygalacturonases. Phytase is the largest enzyme segment in the feed industry, enabling the utilization of phosphorus bound in phytic acid in cereal-based feed, thereby improving phosphorus availability.

Monogastric animals are unable to digest cellulose and hemicellulose in plant-based feeds and they benefit from xylanase and beta-glucanases which aid in the starch's digestion. Proteases break down proteins into amino acids. reducing anti-nutritional factors and enhancing feed efficiency. Microbial enzymes are also used in the paper and pulp industry. With growing awareness of sustainability, the use of microbial enzymes in the paper and pulp industry is increasing in order to mitigate environmental impact by other conventional procedure. Enzymes reduce the processing time, energy consumption and chemical uses while enhancing processes like de-inking, bleaching and waste treatment by improving biological and chemical oxygen demand.



xylanases and ligninases remove lignin and hemicellulases, improving pulp quality. Amylases are used for starch coating, de-inking, paper cleanliness and drainage improvement. Lipases aid in pitch control and de-inking while celluloses apply for de-inking, softening and drainage enhancement as well as recycling printed paper. Laccases serve as a chlorine-free alternative in chemical pulping thereby reducing ozone depleting and acidifying waste. Mannase improves paper brightness by degrading glucomannan.

These enzyme applications make paper production more sustainable and eco-friendly. Microbial enzymes are also used in the leather industry. These leather industry faces significant environmental and health challenges due to the discharge of waste and harmful chemicals like sulphides, lime and amines during various processing stages. Enzymes offer a biodegradable and eco-friendly alternative, improving leather quality while reducing waste and pollution. Key enzymes like proteases, alkaline and neutral lipases and amylases are used across processes such as de-hairing, soaking, bating, degreasing and tanning.

Microbial enzymes in paper and pulp industries

- With growing awareness of sustainability, the use of microbial enzymes in the paper and pulp industry has increased to mitigate environmental impact.
- Enzymes reduce processing time, energy consumption, and chemical usage, while enhancing processes like deinking, bleaching, and waste treatment by improving biological and chemical oxygen demand (BOD and COD).
- **Xylanases** and **ligninases** remove lignin and hemicelluloses, improving pulp quality.
- **Amylases** are used for starch coating, deinking, paper cleanliness, and drainage improvement.
- **Lipases** aid in pitch control and deinking, while **cellulases** are applied for deinking, softening, and drainage enhancement, as well as recycling printed paper.
- **Laccase** serves as a chlorine-free alternative in chemical pulping, reducing ozone-depleting and acidifying waste.
- **Mannases** improve paper brightness by degrading glucomannan. These enzyme applications make paper production more sustainable and eco-friendly.

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Additionally, enzymatic dehairing preserves the hair, reduces reliance on harmful chemicals, and produces softer, more pliable leather. So here, we can see the environment of a leather tanning industry. Enzymes are now being used for a more eco-friendly and sustainable tanning process. Similarly, microbial enzymes are also used in the textile industry, which normally generates significant waste from processes like fabric desizing, bleaching, and dyeing, making it a major contributor to environmental pollution. Enzymes are increasingly used to develop eco-friendly technologies for fiber processing while enhancing product quality.

Microbial enzymes in leather industry

File: Leather tanning in Fes, Morroco. Enzymes are now being used for more eco-friendly and sustainable tanning processes.
[**Credit:** Gagnon, CC BY-SA 3.0, via Wikimedia Commons]

- The leather industry faces significant environmental and health challenges due to the discharge of waste and harmful chemicals like sulfides, lime, and amines during various processing stages.
- Enzymes offer a biodegradable and eco-friendly alternative, improving leather quality while reducing waste and pollution.
- Key enzymes like **proteases** (alkaline and neutral), **lipases**, and **amylases** are used across processes such as dehairing, soaking, bating, degreasing, and tanning.
- Additionally, enzymatic dehairing preserves the hair, reduces reliance on harmful chemicals, and produces softer, more pliable leather.

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Hydrolases such as amylase, cellulase, cutinase, protease, pectinase, and lipase are used for processes like biopolishing, biosourcing, anti-felting of wool, cotton softening, denim finishing, desizing, and synthetic fiber modification. Then there are the oxidoreductases, which include catalase, laccase, peroxidase, and ligninase, which are employed for biobleaching, bleach termination, dye decolorization, and fabric finishing. So in this table, we can see some applications of enzymes in the textile industry. For example, amylase is used for desizing; it is produced in Bacillus. Cellulase is used for cotton softening and denim finishing, produced by Aspergillus and Penicillium. Then we have catalase, which is used for bleach termination, produced by Aspergillus.

Laccase is used for non-chlorine bleaching and fabric dyeing, produced by Bacillus. Then pectate lyase is used for bioscouring, produced by Bacillus. Then comes protease, which is used for the removal of wool fiber scales or degumming of silk, produced by Aspergillus niger. Then we have lipases, which are used for the removal of size lubricants and denim finishing, produced by Candida antarctica. Then we have ligninase, used for wool finishing, produced by Trametes.

And then collagenase, which is also used for wool finishing and produced by Clostridium histolyticum. And then we have cutinase, which is used for cotton scouring and synthetic fiber modification, produced by Pseudomonas, etc. Now let us discuss the microbial enzymes which are used in the detergent industry. These enzymes play a key role in the development of industrial detergents, widely used to remove protein, starch, oil, and fat-based stains, and they include amylase, lipase, cellulase, cutinase, and proteases. Cellulases brighten colors, soften fabrics, and remove small fibers without harming major ones, while cutinases are used for lipolytic cleaning in dishwashing and laundry detergents.

Table showing some applications of enzymes in textile industry

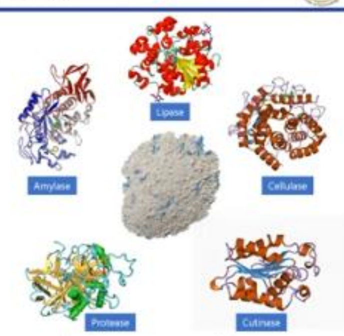
Enzyme	Use	Microorganisms
Amylase	Desizing	*Bacillus* sp., *B. licheniformis*
Cellulose	Cotton softening, denim finishing	*Aspergillus niger, Penicillium funiculosum*
Catalase	Bleach termination	*Aspergillus* sp.
Laccase	Non-chlorine Bleaching, fabric dyeing	*Bacillus subtilis*
Pectate lyase	Bioscouring	*Bacillus* sp., *Pseudomonas* sp.
Protease	Removal of wool fiber scales, degumming of silk	*Aspergillus niger, B. subtilis*
Lipase	Removal of size lubricants, denim finishing,	*Candida antarctica*
Ligninase	Wool finishing	*Trametes versicolor, Phlebia radiata*
Collagenase	Wool finishing	*Clostridium histolyticum*
Cutinase	Cotton scouring, synthetic fiber modification	*Pseudomonas mendocina, Fusarium solani pisi, Thermomonospora fusca*

Data adapted from Singh *et al.*, 2016 59

In dishwashing detergents, proteases and amylases effectively remove protein- and carbohydrate-based food residues. Microbial enzymes are also used for waste management. They play a vital role in facilitating the degradation of toxic pollutants like phenols, aromatic amines, and nitriles from industrial effluents and domestic wastes. They are used individually or in combinations to convert harmful compounds into harmless products through degradation or bioconversion processes. A wide range of enzymes is employed in waste treatment, including amylases, cellulases, lipases, nitrile hydratases, pectinases, and proteases.

Microbial enzymes in detergent industry

- Enzymes play a key role in the development of industrial detergents, widely used to remove protein, starch, oil, and fat-based stains.
- The most commonly used enzymes in detergents are hydrolases: **amylases** target starchy stains, **lipases** remove fatty deposits, and **proteases** digest organic stains like grass, blood, and sweat.
- **Cellulases** brighten colors, soften fabrics, and remove small fibers without harming major ones, while **cutinase** (EC 3.1.1.74) is used for lipolytic cleaning in dishwashing and laundry detergents.
- In dishwashing detergents, proteases and amylases effectively remove protein and carbohydrate-based food residues.



File: Various enzymes present in detergents
[**Credit:** Ayush Jain990, CC BY-SA 4.0, via Wikimedia Commons]

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Oxidoreductases such as laccase, manganese peroxidase, lignin peroxidase, and tyrosinase are particularly effective in detoxifying organic compounds through oxidative coupling. They remove chlorinated phenolic compounds from industrial effluents. Then we have the oxygenases, including monooxygenases and dioxygenases, derived from microbial sources. They exhibit broad substrate specificity and are instrumental in degrading halogenated organic pollutants like herbicides, insecticides, fungicides, and industrial fluids. So with this, we come to the end of today's lecture.

Thank you for your kind attention. Amen.