

Applications of Microbial Enzymes: The Need of an Hour

Ankita Singh¹, PalakVarma², Arpita Singh³, Shuchi⁴, Anakshi⁵, Neha Sharma⁶,
Kajal Rawat⁷, Indra Rautela⁸, Veena Maheshwari⁹, Tanu Allen¹⁰, Narotam Sharma¹¹

How to cite this article:

Ankita Singh, PalakVarma, Arpita Singh, *et al.* / Applications of Microbial Enzymes: The Need of an Hour/Indian J Genet Mol Res. 2023; 12(2):61-74.

Abstract

A growing need for sustainable solutions is one of the primary drivers of the demand for industrial enzymes. One of the most significant and beneficial sources of many enzymes has been and still is the microbial world. Numerous industrial procedures, such as chemical synthesis used to create chemicals and pharmaceuticals, have a number of drawbacks: Lack of enantiomeric specificity for chiral synthesis, low pH, high pressure, high temperature, and low catalytic efficiency. Enzyme research and interest are still advancing, which helps industrial biocatalysis succeed even more. There should be a lot of intriguing discoveries in the field of biotransformation over the coming years. The value of biotechnologically and industrially significant microbial enzymes is the main topic of this study, which comprises 44 papers, including research studies and review articles. Also, it offers novel insights into the micro-organisms that manufacture these enzymes as well as the procedures employed for their purification and separation.

Keywords: Microbial Enzymes; Diagnostics; Lipases; Industry; Bio-Remediation; Medicines; Agriculture; Genetic engineering.

Abbreviations: SPs-Signal peptidases, GI-Gastro-Intestinal, PRP-Platelet Rich Plasma, pNA-Paranitroanilide, CAZymes-carbohydrate active enzymes, AAA+-ATPases associated with diverse cellular activities, BGL- β -Glycosidase, PMSF-phenylmethylsulfonyl fluoride, EDTA-Ethylenediaminetetraacetic acid, CYPs-Cytochromes P450 (*wavelength of absorption maximum of 450 nm*), E.L.C- Enzyme loading capacity, HM-Heavy Metals.

Author Affiliation: ^{1,7,11}DNA Labs-A Centre for Applied Sciences, Dehradun, Uttarakhand 248001, India, ⁸Department of Biotechnology, School of Applied and Life Sciences, Uttaranchal University, Dehradun 248007, Uttarakhand, India, ⁹Bhavan's College (Autonomous), Andheri, Mumbai 400058, Maharashtra, India, ^{9,10}Amity Institute of Biotechnology, Amity University, Noida 201301, Uttar Pradesh, India.

Corresponding Author: Narotam Sharma, DNA Labs-A Centre for Applied Sciences, Dehradun 248007, Uttarakhand, India.

E-mail: sharmanarotam5@gmail.com

Received on: 11.05.2023

Accepted on: 30.06.2023



This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0.

INTRODUCTION

An important family of biomolecules called enzymes is required for the many chemical inter conversions essential to life. They perform the unique function known as biological catalysts, which has the amazing capacity to accelerate reactions more rapidly and effectively. They also quicken all bodily metabolic processes.^{1,2,20} Enzymes have piqued the interest of several businesses since they may be utilised to create products for a number of

uses and have been the focus of numerous studies over the years. Enzymes are found in the human body, animals, plants, and the flora and fauna of microbes. The enzymes produced by bacteria have been found to serve a variety of biological purposes, and as a result, they are utilised in a wide range of applications.^{2-4,24} Additionally, they demonstrate

that a large portion of this product (enzymes) may be produced by suspension culture, which is why microorganisms are considered to be reliable sources for enzyme production and acquisition. In addition to their inherent biochemical diversity, microbial strains can be genetically modified to create desired chemicals.^{5,6,37}

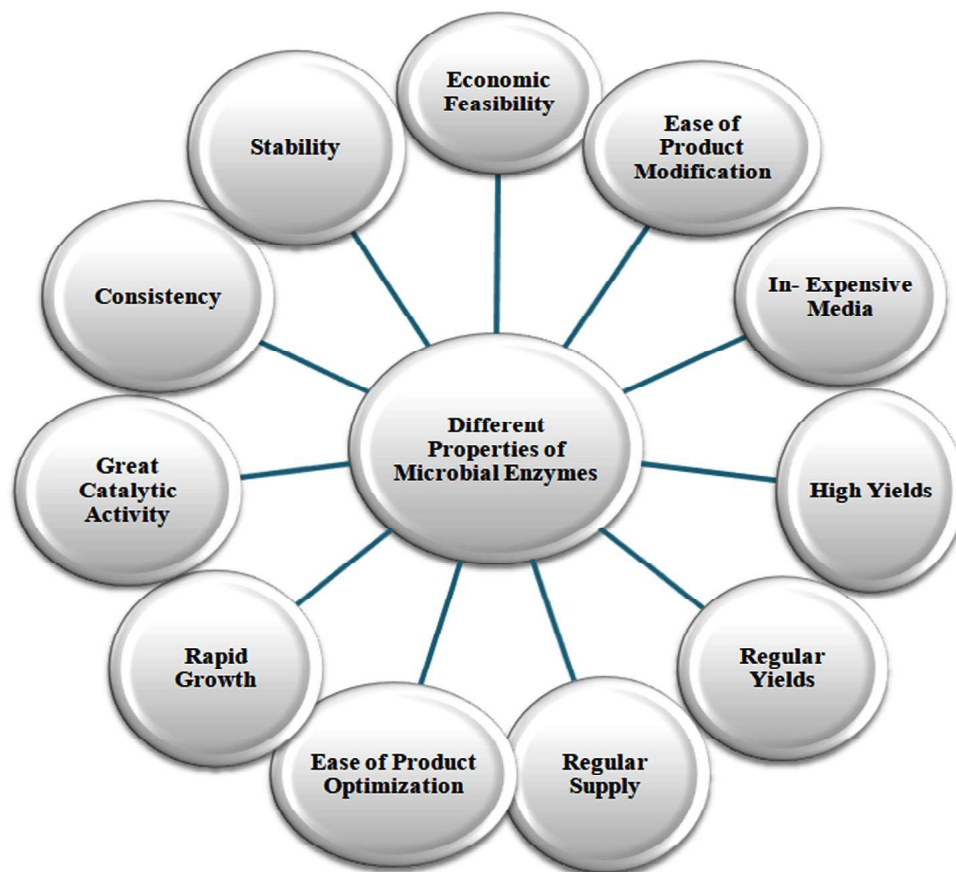


Fig. 1: Versatile Properties of Microbial Enzymes²⁵

Application of Microbial Enzymes in Medical Health and Diagnostics

The importance of medical care and diagnostics cannot be disputed. They are an essential part of our lives since they improve patient care, protect health, and rapidly spot any possible problems. How to employ therapies and drugs to treat and prevent disease is a challenging subject to tackle. Pre-requisites include a diagnosis and fast, easy, and efficient medication. Microbial enzymes fulfil the three E's, and several studies have shown that these enzymes are highly efficient in the fields of medical health and diagnostics. Maude B. Wikstrom *et al.*, 1983, tested 116 strains for

their capacity to produce fibrinogen and fibrin degrading enzymes using blood samples obtained from subjects with angular cheilitis, subgingival plaque on teeth, teeth with infected necrotic pulps, and dental alveoli after surgical removal of mandibular third molars. The most common strains were Actinomyces, Bacteroides, Fusobacterium, Peptococcus, Propionibacterium, and Staphylococcus aureus. Actinomyces, Bacteroides, and Fusobacterium strains with degrading activity are rare. Fibrinogen was degraded by more strains than fibrin, suggesting that a number of proteases may be involved in this process. Furthermore, plasminogen activity was detected in isolates of Clostridium, S. aureus, and Streptococcus

pyogenes. According to the study, bacteria create the enzymes necessary to degrade fibrin and synthesise fibrinogen. The importance and use of microbial enzymes for peptide synthesis, as well as the advancement of biotechnological methods, are highlighted by Kumar Dinesh *et al.* in their 2005 study. According to reports, microbial enzymes can be employed to make peptides and treat various disorders that are hormonal and non-hormonal. Several medical applications of microbial enzymes were outlined by (Irwin W. Sizer *et al.*, 2008). The main take aways are the use of microbial enzymes in blood coagulation, inflammatory treatment, and diagnostics. A description of the microbial enzymes employed in clinical laboratories for quantitative chemical assays and in the treatment of cancer is highlighted in the chapter. Microbial enzymes can be utilised to treat a variety of life threatening illnesses like diabetes, infections, and cardiac arrest when paired with other drugs like antibiotics. Due to their great specificity and quick acting qualities, they can also be utilised to conduct testing. Paul J. Weimer discussed the various medical applications of microbial enzymes. The main take aways are the use of microbial enzymes in blood coagulation, inflammatory treatment, and diagnostics. A description of the microbial enzymes employed in clinical laboratories for quantitative chemical assays and in the treatment of cancer is highlighted in the chapter. Microbial enzymes can be utilised to treat a variety of life threatening illnesses like diabetes, infections, and cardiac arrest when paired with other drugs like antibiotics. Due to their great specificity and quick acting qualities, they can also be utilised to conduct testing. The possibility that ruminant cellulose will be better utilised and that digestive improvements will take place may increase with increases in digestible fodder and enhanced rumen microbial strains and types. Hemerhorst's study provided the first proof of gluten degrading microorganisms linked to the upper GI tract and their function in the digestion of dietary gluten because these salivary microorganisms exhibit glutamine endoprotease activity, which was discovered towards glutamine and proline rich salivary proteins. The main objective was to determine whether gliadins could serve as substrates for the enzymes made by oral microorganisms. The oral microbes proved they could do this by analysing the effects of suspended dental plaque on the proteolytic activity of gliadin derived pNA linked synthetic enzyme substrates. A combination of highly immunogenic gliadin peptides that are produced both naturally and artificially; (33-mer of α 2-gliadin and 26-mer of

gliadin).

Using gliadinzymography, it was also possible to determine the estimated molecular weights and pH activity profiles of the oral enzymes that degrade gliadin. Using liquid isoelectric focusing, the enzymes' total isoelectric points were calculated. Preferentially, the tripeptide YPQ, which is typically present in gluten, is targeted.

As representative gliadin derived substrates, Anaspec in Fremont, California, chemically developed four synthetic analogues: Z-YPQ-pNA, Z-QQP-pNA, Z-PPF-pNA, and Z-PFP-pNA. The bulk of the oral gliadin degrading enzymes were acidic, and it was demonstrated that gliadin degrading enzymes were active over a wide pH range. We observed the proteolytic degradation of gliadin and two other highly immunogenic and protease resistant gliadin peptides. Intriguing genetic parallels between PRPs made from human saliva and gliadins made from wheat were also discovered by the study. In humans, the mouth cavity serves as a breeding habitat for bacteria that facilitate digestion by secreting enzymes that break down gliadin. Therefore, by detoxifying immunogenic gluten peptides, these bacteria and enzymes may result in new treatments for people with celiac disease as well as other disorders. Proteolytic enzymes and treatment procedures help break down gluten. Human oral cavity bacteria and relevant enzymes may enable some novel strategies to detoxify immunogenic gluten peptides and treat patients with celiac disease and other gluten related illnesses. Flint J. Harry and others in 2012 investigated the symbiotic relationship & impact of dietary carbohydrate and prebiotics on human health with the application as a new platform of study of human health, particularly among members of the under studied Firmicutes phylum. The fermentation of complex carbohydrates in the intestine is carried out by a few dominant species among the Bacteroidetes, which owns very large numbers of genes encoding (CAZymes). The microbes in the gut play crucial functions by dissolving complicated substrates. The mammalian intestine is home to bacteria, which have a wide variety of genes and degradative enzymes in their arsenal. It may be possible to identify, characterize, and comprehend the many functions that these bacteria, fungi, and hosts play in metabolic outputs and applications. (Essam Kotb *et al.*, 2013) found that both the methods for measuring fibrinolytic activity and the microorganisms that produce those enzymes were discussed in the literature. It is possible to show how microbial

fibrinolytic enzymes are used in the treatment of myocardial infarction and other cardiovascular diseases. Elizabeth Culp and others discussed the prospects and advancement in the development of medications targeting bacterial proteases, with a particular emphasis on AAA+ proteins family proteolytic complexes. (SPs). The importance of bacterial enzymes, which are essential in the establishment of resistance, was emphasized. Bacterial proteases may prove to be a wealth of new antibiotic targets in the future. Based on their roles

in various metabolic pathways, these enzymes are divided into several categories. Important findings include the enzymatic alteration of intracellular targets, the enzymatic transformation of antibiotics, antibiotic resistance, enzymes, mutant forms, and antibiotics (Table 1 & 2). It is feasible to predict and assess that bacterial enzymes are a fantastic research topic for microbiologists, molecular biologists, and biotechnologists, and employ it in metabolizing antibiotics as drugs.

Table 1: Applications of microbial enzymes in Various industries

Microbial Enzyme	Application
α -Amylase	Baking, brewing, liquefaction of starch enhancing the quality of bread Rice cakes
Glucoamylase	Clarification of fruit juice Manufacturing beer Bread quality improvement High glucose and high fructose syrups
Protease	Brewing Meat tenderization Coagulation of milk Bread quality improvement
Lactase (β -galactosidase)	Lactose intolerance reduction in people Prebiotic food ingredients
Lipase	Cheese flavor enhancement Cheddar cheese production
Phospholipase	Cheese flavor enhancement Production of lipolyzed milk fat
Esterase	Enhancement of flavour and fragrance in fruit juice De-esterification of dietary fibre Production of short chain flavour esters
Cellulase	Animal feed Clarification of fruit juice
Xylanase	Clarification of fruit juice, Beer quality improvement
Pectinase	Clarification of fruit juice
Glucose oxidase	Food shelf life improvement Food flavor improvement
Laccase	Polyphenol removal from wine Baking
Catalase	Food preservation (with glucose oxidase) Removal of hydrogen peroxide from milk prior to cheese production
Peroxidase	Development of flavor, color and nutritional quality of food
α -Acetolactate dehydrogenase	Shortening maturation of beer
Asparaginase	Reduction of formation of acrylamide during baking
Debittering enzymes - naringinase	Removal of bitter taste in fruit juice Wine aroma enhancement

Table 2: Role of Microbial enzymes in Food and Beverage Industries, Chemical Industries and Pharmaceutical Industries.

Microbial Species, Types and Uses		
<i>Saccharomyces cerevisiae</i>	Yeast	Bakery, wine, beer, sake
<i>Saccharomyces carlsbergensis</i>	Yeast	Light beer
<i>Saccharomyces rouxii</i>	Yeast	Soy sauce
<i>Candida milleri</i>	Yeast	Sour dough French bread (sour bread)
<i>Lactobacillus sanfrancisco</i>	Bacteria	Sour dough French bread (sour bread)
<i>Streptococcus thermophilus</i>	Bacteria	Yogurt
<i>Lactobacillus bulgaricus</i>	Bacteria	Yogurt
<i>Propionibacterium shermanii</i>	Bacteria	Swiss cheese
<i>Gluconobacter suboxydans</i>	Bacteria	Vinegar
<i>Penicillium roqueforti</i>	Filamentous fungi	"Roquefort" cheese
<i>Penicillium camemberti</i>	Filamentous fungi	"Camembert" and "Brie" cheeses
<i>Aspergillus oryzae</i>	Filamentous fungi	Saké
<i>Rhizopus sp.</i>	Filamentous fungi	Tempeh
<i>Mucor sp.</i>	Filamentous fungi	Sufu (microbial rennet)
<i>Monascus purpureus</i>	Filamentous fungi	Ang-kak (red rice)
<i>S. cerevisiae</i>	Yeast	Ethanol (from glucose)
<i>Kluyveromyces fragilis</i>	Yeast	Ethanol (from glucose)
<i>Clostridium acetobutylicum</i>	Bacteria	Acetone and butanol
<i>Xanthomonas campestris</i>	Bacteria	Polysaccharides
<i>A. niger</i>	Filamentous fungi	Citric acid
<i>C. glutamicum</i>	Bacteria	l-Lysine, 5-inosinic acid, 5-guanylic acid
Single Cell Proteins (SCP)		
<i>Candida utilis</i>	Yeast	SCP from paper industry waste cultivation
<i>Yarrowia lipolytica</i>	Yeast	SCP from alkane cultivation
<i>Methylophilus methylotrophus</i>	Bacteria	SCP from methane or methanol cultivation
<i>Candida utilis</i>	Yeast	SCP from paper industry waste cultivation
<i>Methylophilus methylotrophus</i>	Bacteria	SCP from methane or methanol cultivation
Vitamins and Enzymes		
<i>Pseudomonas denitrificans</i>	Bacteria	Vitamin B12
<i>Propionibacterium</i>	Bacteria	Vitamin B12
<i>Eremothecium ashbyi</i>	Yeast	Riboflavin
<i>Pseudomonas denitrificans</i>	Bacteria	Vitamin B12
<i>Propionibacterium</i>	Bacteria	Vitamin B12
<i>Trichoderma reesei</i>	Filamentous fungi	Cellulase
<i>S. cerevisiae</i>	Yeast	Invertase
<i>K. fragilis</i>	Yeast	Lactase
<i>S. lipolytica</i>	Yeast	Lipase
<i>Bacillus</i>	Yeast	Protease
<i>Aspergillus</i>	Yeast	Pectinase and protease
<i>Endothia parasitica</i>	Yeast	Microbial rennet
<i>Eremothecium ashbyi</i>	Yeast	Riboflavin
<i>Pseudomonas denitrificans</i>	Bacteria	Vitamin B12
<i>Propionibacterium</i>	Bacteria	Vitamin B12
<i>Trichoderma reesei</i>	Filamentous fungi	Cellulase
<i>S. cerevisiae</i>	Yeast	Invertase

table cont....

<i>K. fragilis</i>	Yeast	Lactase
<i>S. lipolytica</i>	Yeast	Lipase
<i>Bacillus</i>	Yeast	Protease
<i>Aspergillus</i>	Yeast	Pectinase and protease
<i>Endothia parasitica</i>	Yeast	Microbial rennet
<i>Aspergillusoryzae</i>	Filamentous fungi	Amylase
<i>A. niger</i>	Filamentous fungi	Glucoamylase
<i>Trichoderma reesei</i>	Filamentous fungi	Cellulase
<i>S. cerevisiae</i>	Yeast	Invertase
<i>K. fragilis</i>	Yeast	Lactase
<i>S. lipolytica</i>	Yeast	Lipase
<i>Bacillus</i>	Yeast	Protease
<i>Aspergillus</i>	Yeast	Pectinase and protease
<i>Endothia parasitica</i>	Yeast	Microbial rennet
<i>Leuconostoc mesenteroides</i>	Bacteria	Dextran
<i>X. campestris</i>	Bacteria	Xanthan gum
<i>Blakesleatrispora</i>	Filamentous fungi	β -Carotene
<i>Phaffiarhodozyma</i>	Yeast	Astaxanthine
<i>Penicillium chrysogenum</i>	Filamentous fungi	Penicillin
<i>Cephalosporium acremonium</i>	Filamentous fungi	Cephalosporin
<i>Streptomyces</i> sp.	Bacteria	Amphotericin B, kanamycin, neomycin, streptomycin, tetracycline, etc.
<i>Bacillus brevis</i>	Bacteria	Gramicidin S
<i>Bacillus subtilis</i>	Bacteria	Bacitracin
<i>Bacillus polymyxa</i>	Bacteria	Polimixin B
<i>Rhizopusnigricans</i>	Filamentous fungi	Steroids transforming
<i>Arthrobacter simplex</i>	Bacteria	Steroids transforming
<i>Mycobacterium</i>	Bacteria	Steroids transforming
<i>E. coli</i> ^a	Bacteria	Insulin, human growth, hormone, somatostatin, and interferon
<i>Bacillus thuringiensis</i>	Bacteria	Bioinsecticide
<i>Bacillus popilliae</i>	Bacteria	Bioinsecticide
<i>Bacillus brevis</i>	Bacteria	Gramicidin S
<i>Bacillus subtilis</i>	Bacteria	Bacitracin
<i>Bacillus polymyxa</i>	Bacteria	Polimixin B
<i>Rhizopusnigricans</i>	Filamentous fungi	Steroids transforming
<i>Arthrobacter simplex</i>	Bacteria	Steroids transforming
<i>Mycobacterium</i>	Bacteria	Steroids transforming
<i>E. coli</i> ^a	Bacteria	Insulin, human growth, hormone, somatostatin, and interferon
<i>Bacillus thuringiensis</i>	Bacteria	Bioinsecticide
<i>Bacillus popilliae</i>	Bacteria	Bioinsecticide
<i>E. coli</i> ^a	Bacteria	Insulin, human growth, hormone, somatostatin, and interferon

Application of Microbial Enzymes in Pharmaceutical, Food, Textiles, Agrochemical Industries

Food and Textiles Industry: A bacterial strain

that secreted a proteolytic enzyme in an organic solvent medium was identified as *Pseudomonas aeruginosa*, according to a 1995 study by Hiroyasu Ogino *et al.* A high rate proteolytic enzyme producer was initially isolated using the conventional

method, and then an organic solvent tolerant microbe was selected from this group of high rate proteolytic enzyme producers to create the strain. The culture's supernatant's proteolytic activity was examined in organic solvents, and it was found to be stable. The stability of the enzyme was essentially the same when there were no organic solvents present when the values of the partition coefficient's logarithm ($\log P$) were equal to or higher than 3.2.³ When various organic solvents are available, the enzyme and solvent stable bacterium of this strain can both be employed as catalysts. With the use of recombinant DNA technology, special enzymes that are appropriate for specific food processing scenarios may now be produced. In a study by Zofia S. Olempska Beer *et al.*, the manufacture and safety assessment of enzyme preparations, the generation of recombinant production strains, approaches for improving enzyme properties, and the safety of microorganisms used as hosts for enzyme encoding genes are briefly discussed by removing native genes encoding extracellular proteases, it is possible to construct microbial and fungal strains specifically for the production of enzymes and modify them to reduce or eliminate their capacity to make toxic secondary metabolites. This increases the yield of the enzymes produced. The potential for oxidative enzymes with high efficiency, inherent stereo and regioselectivity, and stereo and regioselectivity to operate as biocatalysts for different biotechnological processes was highlighted, according to (F. Sima Sariaslani *et al.*, 20080). It is obvious that microbial enzymes have uses in a variety of businesses, including those that specialise in genetic engineering.

Fermentation Industries: According to a 2016 study by Giyatmi *et al.*, a variety of enzymes are examined along with their relevance, including natural fish enzymes, fermented fish products, microbial enzymes, and muscle tissue enzymes. It is shown how the fermentation process works with microbial enzymes derived from fish. In addition, (Bowen Wang *et al.*, 2010) reported that they investigated the activity of enzymes from *Aspergillus*, *Rhizomucor*, and *Rhizopus* and used metaproteomics to find 51 carbohydrate hydrolases in the fermentation of Chinese spirits. The prospect of conducting additional studies on fermented fish is also explored. Dark fermentation is a technique utilised by Prakash K. Sarangi *et al.*, 2020 to create bio hydrogen from waste biomass. The key finding of dark fermentation showed how well microbial enzymes can ferment biomass to produce biofuels from waste. They talked about various biological hydrogen generating systems as well as process

parameters. The carefully selected bacterial and fungal strains can be used in industrial manufacture. Because amylases are essential for turning starches into oligosaccharides. The study maintains starch as a significant element because it is a staple food for humans and a storage product of numerous crops, including wheat, tapioca, and maize. The emphasis of the article is on the structural and functional characteristics, distribution, and production of amylase using submerged fermentation and solid state fermentation methods to illustrate its properties, including thermo stability, pH, and purification using chemical and physical factors. Amylase is one of the primary enzymes used in industrial processes like food production, fermentation, etc. A wide range of plants, animals, and microorganisms can produce amylases. Microbial pectinases are a group of enzymes that have potential applications across a range of sectors, according to the literature. These enzymes are widely used in a range of industries, including the wine, food, and paper industries, and are thought to be environmentally safe. The extracellular thermostable alkaline protease was demonstrated to be produced from the A10 strain by (Yilmaz Bahar *et al.*, 2015). The A10 strain was purified 1.38-fold with 9.44% efficiency using DE52 anion exchange chromatography and ammonium sulphate precipitation dialysis. The study's guiding principles are the purification and characterization of the alkaline protease enzyme from the thermophilic bacterium *B. licheniformis* A10. According to investigation, A10 protease enzyme displays excellent activity and stability at high temperatures and pH levels and can be employed in a number of industrial processes.⁹ Noura Raddadi *et al.*, (2015) discussed the ecology and most current biotechnological applications of microbial extremophiles, extremozymes, and extremolytes. Microorganisms that produce extremozyme and extremolytes are valuable resources for the development of a bio-based economy.

Pharmaceutical Industries: New studies into novel bacteria that can be used to produce amylase would be very advantageous for the pharmaceutical, starch based, and therapeutic industries Including Daljit Singh Arora, In order to better understand the mechanisms underlying the reactions of interest and to maximise laccase's catalytic activity for usage in a variety of biotechnology applications, the study uses multiple physiochemical parameters to interpret laccase synthesis. Microbial enzymes can be utilised to modify synthetic chemicals for pharmaceuticals and agricultural applications since chiral intermediates and fine chemicals are

highly sought after by the pharmaceutical and agrochemical industries. (Patel, Ramesh N., 2002) detailed the microbial and enzymatic processes in his study and synthesised chiral intermediates for the treatment of cancer, viruses, hypertension, melatonin receptor agonists, high cholesterol, and Alzheimer's disease using enzymes derived from bacteria.

Chemical and Agro Chemical Industries: The best substitute (BGL) that the fungus creates, according to Tiwari Pragya *et al.*, are the microbial enzymes. *Trichoderma* collaborates to completely hydrolyze cellulose in order to produce biofuel as a solution to the energy issue. Numerous modification strategies, such as the development of glucose tolerant (BGL) and external delivery in conjunction with other celluloses, are used in the bulk of current applications for (BGL). A few major microbial enzymes are reviewed by Nigam Singh Poonam in a study done in 2013 along with their distinctive characteristics and commercial relevance. Over the years, scientists have isolated and documented a range of specialised bacteria from extreme sources to see if these microbes are capable of biosynthesizing particular enzymes. To produce high-quality enzyme preparation on a large scale for various commercial uses, a varied range of enzymes are being synthesised employing chosen microorganisms with specific properties. Biotechnology is starting to be seen as a solution to several global issues. The study of microbial enzymes and the techniques used to make them have improved large yields of enzymes with useful specific qualities, such as thermal stability, tolerance to high temperatures, and stability in acidic or alkaline conditions. Mohamed A. Hassan and others in 2015 studied that Both the *Bacillus amyloliquefaciens* MA20 and *Bacillus subtiles* MA21 soil isolated strains were discovered in their study. Study of their morphological, biochemical, and molecular characteristics revealed that they were sensitive to PMSF and that they produced serine proteases that were stable in organic solvents and only slightly inhibited by EDTA. Because the two proteases created in the study are metal enhanced and stable in the majority of organic solvents, they can be exploited in biotechnological applications like the wool industry. *Aspergillus Niger*, a fungus that originated in a maritime environment, was the subject of research by Ghada E. A. Awad *et al.* in 2015. In order to create gel beads that were biocatalytically active, *Aspergillus Niger*'s induced naringinase was immobilised. Immobilization and E.L.C., chemical, and physical characterisation optimisation were the main

subjects of the investigation. The immobilisation process greatly improved the enzyme's thermal stability, which is helpful in the food sector. On a commercial as well as an industrial level, these results are quite beneficial. The findings paves the way for further investigation and aids in the production of immobilised naringinase for use in applications beyond marketing and industry. Girvan M. Hazel and others (2016) said that the role of wild type and modified P450s in the production of major chemicals, including drugs and drug metabolites, steroids, antibiotics, and members of the CYPs super family, is discussed in the article. It is clear that CYPs are crucial for synthetic biology. Vildan Yildirim and others (2017) in a study done on the extracellular thermostable alkaline serine protease enzyme was biochemically isolated, characterised, and purified 4.85 and 17.32 times, with a yield of 26.9 and 19.56%, respectively, using DE52 anion exchange and probond affinity chromatography. It was revealed that the enzyme activity was retained at levels more than 70% and 55%, respectively, in the presence of organic solvents and commercial detergents. As can be shown, pure protease enzyme may be preferred for commercial applications that require long-term stability at high temperatures. *A. pallidus* C10 alkaline protease is a great detergent additive in a number of commercial and biotechnological fields, particularly the detergent industry. The yields of fourteen model enzymes in *E. coli* cultures cultured in fresh, ambiguous media and in complicated media were rigorously compared in the study by Lukas Chrast *et al.* (2017). In conclusion, the experimental innovative semi-defined medium offers shake flask protein yields without the need for costly bioreactor purchases. All of the studied enzymes expressed in enzymatic fed batch like cultures showed improvements in the volumetric yields without significantly affecting the structure, stability, or activity of the enzymes. Rodrigues, Ronivaldo da Silva in 2017 demonstrated the biochemical and biotechnological characteristics of the seven various types of catalytic types with an emphasis on new studies, production, catalysis, and uses of these enzymes. The discovery makes it simpler to develop new enzyme based goods with applications in food, medicine, basic science, and bioactive peptides. Among others, Abdul Razzaq (2019) Compared various proteases and contemporary issues with industrial production and use are the review's main focuses. Microbial proteases might be offered economically and commercially on a global scale if these problems were solved. For industries with widespread use

(Table 3), like textile, pharmaceuticals, leather, food, and detergent, the interpretation is fully dependent on microbial enzymes.

Table 3: Industrial applications of microbial enzymes

Industry	Enzyme	Function	Microorganisms
Dairy	Acid proteinase	Milk coagulation	<i>Aspergillus</i> sp.
	Neutral proteinase	Faster cheese ripening, debittering	<i>Bacillus subtilis</i> , <i>A. oryzae</i>
	Lipase	Faster cheese ripening, flavor customized cheese,	<i>Aspergillusniger</i> , <i>A. oryzae</i>
	Lactase (β -galactosidase)	Lactose reduced milk and whey products	<i>Escherichia coli</i> , <i>Kluyveromyces</i> sp.
	Aminopeptidase	Faster cheese ripening	<i>Lactobacillus</i> sp.
	Catalase	Cheese processing	<i>Aspergillusniger</i>
	Transglutaminase	Protein cross linking	<i>Streptomyces</i> sp.
	Baking	Amylase	Flour adjustment, bread softness
Maltogenic α -Amylase		Enhance shelf life of breads	<i>Bacillus stearothermophilus</i>
Xylanase		Dough conditioning	<i>Aspergillusniger</i>
Lipase		Dough stability and conditioning	<i>Aspergillusniger</i>
Glucose oxidase		Dough strengthening	<i>Aspergillusniger</i> , <i>Penicilliumchrysogenum</i>
Transglutaminase		Laminated dough strength	<i>Streptoverticillium</i> sp., <i>streptomyces</i> sp.
Beverage	Pectinase	Depectinization	<i>Aspergillusoryzae</i> , <i>Penicilliumfuniculosum</i>
	Glucose oxidase	Oxygen removal from beer	<i>Aspergillusniger</i>
	Cellulase	Fruit liquefaction	<i>Aspergillusniger</i> , <i>Trichodermaatroviride</i>
	α -Amylase	Starch hydrolysis	<i>Bacillus</i> , <i>Aspergillus</i>
	β -Amylase	Starch hydrolysis	<i>Bacillus</i> , <i>Streptomyces</i> , <i>Rhizopus</i>
	β -Glucanase	Restrict haze formation	<i>Bacillus subtilis</i> , <i>Aspergillus</i> spp.
	Protease	Restrict haze formation	<i>Aspergillusniger</i>
	Pullulanase	Starch saccharification	<i>Bacillus</i> sp., <i>Klebsiella</i> sp.
	Naringinase	Debittering	<i>Aspergillusniger</i>
	Limoninase	Debittering	<i>Aspergillusniger</i> , <i>A. oryzae</i>
	Aminopeptidases	Protein breakdown during mashing	<i>Lactobacillus brevis</i> , <i>L. plantarum</i>
Animal feed	Phytase	Hydrolyze phytic acid to release phosphorous	<i>Aspergillusniger</i>
	Xylanase	Enhanced digestibility of starch	<i>Aspergillus</i> sp., <i>Bacillus</i> sp.
	β -glucanase	Digestive aid	<i>Aspergillusniger</i>

table cont....

Pulp and paper	Lipase	Pitch control	<i>Candida Antarctica</i>
	Protease	Biofilm removal	<i>Bacillus subtilis</i>
	Amylase	Deinking, drainage improvement	<i>Bacillus licheniformis</i>
	Xylanase	Bleach boosting	<i>Trichodermareesei,</i> <i>Thermomyceslanuginosus,</i> <i>Aureobasidiumpullulans</i>
	Laccase	Non-chlorine bleaching, delignification	<i>Bacillus subtilis</i>
	Cellulase	Deinking, drainage improvement	<i>Bacillus sp., Aspergillusniger</i>
Polymer	Lipase	Polycondensation, ring-opening polymerization of lactones, carbonates	<i>Candida Antarctica</i>
	Laccase	Polymerization of bisphenol A	<i>TrametesHirsuta</i>
	Glucose oxidase	Polymerization of anilines	<i>Aspergillusniger,</i> <i>Penicilliumchrysogenum</i>
	Transglutaminase	Crosslinking of protein	<i>Streptomyces mobaraensis</i>
	Tyrosinase	Polymerization of lignin and chitosan	<i>Trichodermareesei</i>
Detergent	Amylase	Carbohydrate stain removal	<i>Aspergillus sp., Bacillus subtilis</i>
	Lipase	Fat stain elimination	<i>Aspergillusoryzae, A. flavus,</i>
	Protease	Protein stain removal	<i>Aspergillusoryzae, Bacillus subtilis</i>
	Cellulase	Color clarification	<i>Aspergillusniger, Bacillus sp.</i>
	Cutinase	Triglyceride removal	<i>Fusariumsolani f. pisi</i>
	Mannanase	Mannan spot removal	<i>Bacillus sp.</i>
Leather	Alkaline protease	Dehairing, bating	<i>Alcaligenesfaecalis</i>
	Neutral Protease	Dehairing, soaking	<i>Aspergillusniger, A. flavus, Bacillus subtilis</i>
	Lipase	Degreasing	<i>Aspergillusoryzae, A. flavus,</i>
	Amylase	Fiber splitting	<i>Aspergillus sp., Bacillus subtilis</i>
Cosmetics	Superoxide dismutase	Free radical scavenging, skin care	<i>Corynebacterium Glutamicum,</i> <i>Lactobacillus plantarum</i>
	Protease	Removal of dead skin	<i>Aspergillusniger, A. flavus, Bacillus subtilis</i>
	Endoglycosidase	Teeth and gum tissue care	<i>Mucorhiemalis</i>
	Laccase	Hair dye	<i>Bacillus subtilis, Trametesversicolor</i>
	Lipase	Skin care	<i>Aspergillusoryzae, A. flavus</i>
Organic synthesis	Lipase	Synthesis of pharmaceuticals, polymers, biodiesels, biosurfactants	<i>Aspergillusoryzae, A. flavus</i>
	Glycosyltransferase	Synthesis of oligosaccharides	<i>Bacillus sp.</i>

table cont.....

Waste management	Nitrile hydratase	Synthesis of acrylamide, butyramide, nicotinamide	<i>Rhodococcusrhodochrous</i> PA-34, <i>Bacillus</i> sp. APB-6
	Glucose isomerase	Production of High fructose corn syrup	<i>Corynebacterium</i> sp., <i>streptomycesmurinus</i>
	Acyltransferase	Synthesis of hydroxamic acids	<i>Bacillus</i> sp. APB-6
	Laccase	Production of textile dyes, cosmetic pigments, flavor agents, and pesticides	<i>Trametesversicolor</i> , <i>Bacillus subtilis</i>
	Amidase	Degradation of nitriles containing wastes	<i>Rhodococcuserythropolis</i>
	Amylase	Bioremediation of vegetables wastes	<i>B. licheniformis</i> , <i>Aspergillus</i> sp.
	Amyloglucosidase	Starch hydrolysis for bioremediation	<i>Aspergillusniger</i>
	Lipase	Degradation of crude oil hydrocarbons	<i>Aspergillusoryzae</i> , <i>Candida tropicalis</i>
	Nitrile hydratase	Degradation of nitriles containing wastes	<i>Rhodococcus</i> sp.
	Protease	Bioremediation of keratinic wastes	<i>Chrysosporium keratinophilum</i>
	Laccase	Degradation of waste containing olefin unit, polyurethane and phenolic compounds	<i>Trametesversicolor</i>
	Cutinase	Degradation of plastics, Polycaprolactone	<i>Fusariumsolani</i> f. <i>pisi</i>
	Manganese peroxidase	Degradation of phenolic compounds	<i>Phanerochaetechrysosporium</i> , <i>Coprinuscinereus</i>
	Lignin peroxidase	Degradation of phenolic compounds	<i>Phanerochaetechrysosporium</i> , <i>Coprinuscinereus</i>
	Oxygenase	Degradation of halogenated contaminants	<i>Pseudomonas</i> sp., <i>Rhodococcus</i> sp.

Application of Microbial Enzymes in Agricultureenvironmental Monitoring, Bio-Remediation

According to Naoto Ogawa's 2003 statement, a lot of study has been done on the genetic relationships, regulation strategy, function, and evolution of microbial genes and enzymes. The microbial degradation of chlorobenzoates, chlorocatechols, and chlorophenoxyacetic acids is discussed in this review with emphasis on the genetic structure, the regulation of gene expression, and evolutionary considerations. Numerous chlorinated compounds are produced by the chemical industry, and the release of such substances into the environment has led to serious environmental problems. One of the causes of this contamination is the inability of natural bacteria to efficiently break down manmade chlorinated compounds. Microorganisms are remarkably adaptable to environmental changes, which has led to the development of genes that specify the breakdown of chlorinated chemicals to

varying degrees. It is evident that the environmental contamination brought on by the chemical industry's production of numerous chlorinated compounds and subsequent release into the environment has made it challenging for natural microbes to efficiently break down manufactured chlorinated chemicals. Genetic engineering, however, can enable them to efficiently break down manmade chlorinated chemicals, which are in charge of global pollution. With an emphasis on this procedure, (Wanpeng Wang *et al.*, 2013) described how bacteria now metabolise long-chain alkanes aerobically. The regulation of alkane degrading genes, early oxidation, chemo taxis to alkanes, and other factors are all factors that the authors take into consideration. Alkanes and hydrocarbons are two contaminants found in the atmosphere. There are some yeasts, filamentous fungi, and microorganisms that are particularly good at degrading alkanes. It is possible to increase the likelihood of removing contaminants based on alkanes that are harmful to living systems by studying the microbial

activity of certain microorganisms as well as the fundamental mechanisms of bacterial alkane dependent chemotaxis, alkane transport, and gene expression regulation. (Gajendiran Anudurga., 2016) The examination into the degradation of DPE by *A. clavatus* was seen during a period of 90 days of incubation in aqueous medium. Changes in polyethylene weight, CO₂ development using the Strum test, infrared spectra, and infrared spectra all showed signs of degradation. Morphological changes were also discovered by SEM and AFM studies. *A. Clavatus* is a suitable option for the degradation of LDPE, however more research is needed to support the applications. Zhang W, Lin Z, and others (2020) The main areas of research attention were the screening of lindane (Pesticides)degrading strains and the analysis of degradation products in lab settings. The supporting technologies and procedures for microbial degradation based bioremediation ought to be created and widely applied. By producing extracellular and intracellular enzymes that break down lindane into less hazardous and non-toxic chemicals, the study found that microbial metabolism can effectively break down lindane. Similar to this, specific bacterial strains may influence how various pesticides and insecticides degrade. For instance, lindane degrades differently depending on the microorganisms that break it down. The breakdown of lindane is known to involve several strains of *Microbacterium* sp. P27, *Paracoccus* sp., and *Phragmites karka. et al.* Haldar Shyamalina *et al.*, 2020). The essay examines Abhijeet Al's (2017) studies on xylanase control and their potential industrial applications, with a focus on pulp bioleaching and the reduction of environmental pollution, as well as the microbial enzyme complex that completely breaks down xylan down, and discusses the accumulation of HM in aquatic environments in India. Our knowledge of the roles and repertoire of the enzyme, which is found in numerous bacteria, will grow as a result of research on extreme temperature enzymes and cutting edge technologies like genome sequencing.

CONCLUSION

Applications for microbial enzymes can be found in a wide range of industries, such as chemical, fermentation, agriculture, medicines, and food manufacturing. Recombinant enzymes have been expressed in bacteria, filamentous fungus, and yeasts; choosing the right expression systems is crucial for the rate of enzyme production. The

benefits of these species have led to an increase in the number of biotechnological applications. Nevertheless, physiological effects make it challenging to produce high level expression of recombinant enzymes. The natural enzymes have drawbacks, particularly in industrial settings include limited activity, stability, and catalytic effectiveness. To get around these restrictions, techniques like site directed mutagenesis, truncation, and terminal fusion have been employed. One thing is clear from all the research done over the years: microbial enzymes are extremely important both physiologically and commercially. They have both synthetic and degradative qualities. These can be found in microorganisms, plants, and animals. However, microorganisms are the preferable supply because they are a treasure trove of different enzymes. The hunt for better strains of the microorganisms employed in the industry and an irreplaceable resource for biotechnological developments both depend heavily on biodiversity. Because enzymes are environmentally neutral and don't produce greenhouse gases or energy intensive waste products, they are replacing chemicals in many industrial production processes. Microbial sources of enzyme production are the most preferred source for industrial enzyme production because the microbes are easily accessible, they grow at a very fast rate, and they can be genetically modified to produce enzymes that can perform optimally at different industrial production conditions. This helps the microbes survive the harsh production conditions and meet the ever increasing demand for enzymes in many industries. Microbial enzymes are exceedingly adaptable and have applications in a wide range of industries, including those in the textile, leather, paper and pulp, research and development, pharmaceutical, agricultural, detergent, waste, biorefineries, photography, and food sectors. People have always been selfish and are constantly seeking for better ways to live. These microbial enzymes' research, efforts, and results are regarded as one of the most promising future options because they are crucial to the diagnosis, therapy, biochemical examination, and monitoring of several terrible conditions. In a very short period of time, modern biotechnology has quickly developed. In a very short period of time, modern biotechnology has quickly developed from a scientific curiosity to a lucrative industry. Because to break throughs in microbiology and biotechnology, it is now possible to create these magnificent enzymes, which will also make it simpler for us to use them in ways that will enhance our quality of life and the environment we live in.

REFERENCES

1. Maude B. W, 1983, Fibrinogenolytic and Fibrinolytic Activity in Oral Microorganisms, *Journal of Clinical Microbiology*, Gunnar Dahltn and lindeanders 1983, Vol. 17, No. 5, p. 759-767.
2. H Ogino, 1995, Organic Solvent-Tolerant Bacterium Which Secretes an Organic Solvent-Stable Proteolytic Enzyme Applied and Environmental Microbiology Dec, K Yasui, T Shiotani, T Ishihara, H Ishikawa 1995, 61 (12) 4258-4262.
3. Patel RN, 2003, Microbial/enzymatic synthesis of chiral pharmaceutical intermediates. *Curr Opin Drug Discov Devel.* 2003;6(6):902-920.
4. Ogawa N., 2003, Microbial genes and enzymes in the degradation of chlorinated compounds. Miyashita K, Chakrabarty AM 2003, *Chem Rec.*; 3(3):158-171. doi:10.1002/tcr.10059.
5. Kumar D, Bhalla TC. Microbia, 1 proteases in peptide synthesis: approaches and applications. *Appl Microbiol Biotechnol.* 2005; 68(6):726-736. doi:10.1007/s00253-005-0094-7.
6. Zofia S., 2006, Food-processing enzymes from recombinant microorganisms--a review, *Regulatory Toxicology and Pharmacology*, Olempska-Beer Robert I. Merker Mary D. Ditto Michael J. DiNovi, 2006, volume 45, Issue 2 July 2006, Pages 144-158.
7. Weimer, Paul, 2008, Cellulose Degradation by Ruminant Microorganisms, *Critical Reviews in Biotechnology*, VL - 12, EP - 223 SP - 189, PY - 2008/09/27, 10.3109/07388559209069192.
8. De Souza, Paula Monteiro 2010. "Application of microbial α -amylase in industry - A review." *Brazilian journal of microbiology* : [publication of the Brazilian Society for Microbiology], P erola de Oliveira Magalh aes vol. 41,4 (2010): 850-61. doi:10.1590/S1517-83822010000400004.
9. Sariaslani FS. Microbial enzymes for oxidation of organic molecules. *Crit Rev Biotechnol.* 1989; 9(3):171-257. doi:10.3109/07388558909036736.
10. Irwin W. Sizer, 2008, Medical Applications of Microbial Enzymes *Advances in Applied Microbiology*, Volume 15, 1972, Pages 1-11.
11. Daljit Singh Arora *et al.*, 2009, Ligninolytic Fungal Laccases and Their Biotechnological Applications, *Applied Biochemistry and Biotechnology*, Sharma Kumar Rakesh 2009, *Appl Biochem Biotechnol* (2010) 160:1760-1788 DOI 10.1007/s12010-009-8676-y.
12. Helmerhorst EJ, 2010, Discovery of a novel and rich source of gluten-degrading microbial enzymes in the oral cavity. *PLoS One*. Zamakhchari M, Schuppan D, Oppenheim FG 2010;5(10):e13264. Published 2010 Oct 11. doi:10.1371/journal.pone.0013264.
13. Harry J. Flint., 2012. Microbial degradation of complex carbohydrates in the gut, *Gut Microbes*, Karen P., Sylvia H. Duncan, Petra Louis & Evelyne Forano 2012, Volume 3, 2012 - Issue 4, Pages 289-306.
14. Wang W, 2013, Enzymes and genes involved in aerobic alkane degradation. *Frontier Microbiology*, Shao Z., 2013; 4:116. Published 2013 May 28. doi:10.3389/fmicb.2013.00116.
15. Rachel N. Austin, 2013, Microbial Enzymes That Oxidize Hydrocarbons, *Frontiers in Microbiology* callahgan.b.ammy, doi:10.3389/fmicb.2013.0038.
16. Tiwari pragya, 2013, β -Glucosidases from the Fungus *Trichoderma*: An Efficient Cellulase Machinery in Biotechnological Applications, *BioMed Research International*, Misra B. N. 2 and Sangwa Neelam S., Volume 2013 | Article ID 203735 | 10 pages | <https://doi.org/10.1155/2013/203735>.
17. Nigam Singh Poonam, 2013, Microbial Enzymes with Special Characteristics for Biotechnological Applications, *Biomolecules*, vol. 3, 3 597-611. 23 Aug. 2013, doi:10.3390/biom3030597.
18. Essam Kotb, 2013, Activity assessment of microbial fibrinolytic enzymes, *Applied Microbiology and Biotechnology*,. 2013;97(15):6647-6665. doi:10.1007/s00253-013-5052-1.
19. Mohamed A. Hassan, 2013, Production and Characterization of Keratinolytic Protease from New Wool-Degrading *Bacillus* Species Isolated from Egyptian Ecosystem, *BioMed Research International*, Bakry M. Haroun, Amro A. Amara, and Ehab A. Serour, Volume 2013 | Article ID 175012 | 14 pages | <https://doi.org/10.1155/2013/175012>.
20. WIM J. QUAX.
21. Awad, G.E.A., Abd El Aty, A.A., Shehata, A.N. *et al.* Covalent immobilization of microbial naringinase using novel thermally stable biopolymer for hydrolysis of naringin. *3 Biotech* 6, 14 (2016). <https://doi.org/10.1007/s13205-015-0338-x>.
22. Garg G, Singh A, Kaur A, Singh R, Kaur J, Mahajan R. Microbial pectinases: an ecofriendly tool of nature for industries. *3 Biotech.* 2016;6(1):47. doi:10.1007/s13205-016-0371-4.
23. Yilmaz B, Baltaci MO, Sisecioglu M, Adiguzel A. Thermotolerant alkaline protease enzyme from *Bacillus licheniformis* A10: purification, characterization, effects of surfactants and organic solvents. *J Enzyme Inhib Med Chem.* 2016;31(6):1241-1247. doi:10.3109/14756366.2015.1118687.
24. Raddadi, Noura & Cherif, Ameer & Daffonchio, Daniele & Neifar, Mohamed & Fava, Fabio. (2015). Biotechnological applications of extremophiles, extremozymes and extremolytes. *Applied microbiology and biotechnology*. 99. 10.1007/s00253-015-6874-9.
25. Gajendiran A, Krishnamoorthy S, Abraham J. Microbial degradation of low-density polyethylene (LDPE) by *Aspergillus clavatus* strain JASK1 isolated from landfill soil. *3 Biotech.* 2016;6(1):52.

- doi:10.1007/s13205-016-0394-x.
26. Giyatmi, 2016, Enzymes in Fermented Fish, *Advances in Food and Nutrition Research*, Irianto H.E Volume 80, 2017, Pages 199-216.
 27. Hazel M Girvan, 2016, Applications of microbial cytochrome P450 enzymes in biotechnology and synthetic biology, *Current opinion in Chemical Biology*, Munro W Andrew, Volume 31, April 2016, Pages 136-145.
 28. Timo Stressler, 2016, I, Kuhn A, Fischer L. Detection, production, and application of microbial arylsulfatases. *Microbiol Biotechnol.* 2016; 100(21):9053-9067. doi:10.1007/s00253-016-7838-4.
 29. Singh, Rajendra *et al.* "Microbial enzymes: industrial progress in 21st century." *3 Biotech* vol. 6,2 (2016): 174. doi:10.1007/s13205-016-0485-8.
 30. Jamil Shafi., 2017, *Bacillus* species as a versatile weapons for plant pathogens: a review, *Biotechnology & Biotechnological Equipment*, |, Tianhui, Mingshan ji, 2017, Pages 446-459.
 31. Sahay, H., Yadav, A.N., Singh, A.K. *et al.* Hot springs of Indian Himalayas: potential sources of microbial diversity and thermostable hydrolytic enzymes. *3 Biotech* 7, 118 (2017). <https://doi.org/10.1007/s13205-017-0762-1>.
 32. Elizabeth Culp *et al.*, 2017.
 33. Abhishek *et al.*, 2017 Microbial xylanases and their industrial application in pulp and paper biobleaching: a review, *3 Biotech*.
 34. Lukas Chrast *et al.*, Gram-scale production of recombinant microbial enzymes in shake flasks, *FEMS Microbiology Letters*, 2017.
 35. Ronivaldo Rodrigues da Silva, *Bacterial and Fungal Proteolytic Enzymes: Production, Catalysis and Potential Applications*. *Applied Biochemistry and Biotechnology*, 2017.
 36. Vildan Yildirim., *et al*, *Bacterial Enzymes and Antibiotic Resistance*, Actanaturae, 2018.
 37. Abdul Razzaq *et al.*
 38. Kumar S. Swaroop, *Fibrinolytic Enzymes for Thrombolytic Therapy Therapeutic Enzymes: Function and Clinical Implications*, 2019, Sabu Abdulhameed, Volume 1148 ISBN: 978-981-13-7708-2.
 39. Okpara, Michael. (2022). Microbial Enzymes and Their Applications in Food Industry: A Mini-Review. *Advances in Enzyme Research*. 10. 23-47. 10.4236/aer.2022.101002.
-
-
-