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MINI-REVIEW

Bioprospecting of microbial enzymes: current trends in industry and healthcare

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Abstract

Microbial enzymes have an indispensable role in producing foods, pharmaceuticals, and other commercial goods. Many novel enzymes have been reported from all domains of life, such as plants, microbes, and animals. Nonetheless, industrially desirable enzymes of microbial origin are limited. This review article discusses the classifications, applications, sources, and challenges of most demanded industrial enzymes such as pectinases, cellulase, lipase, and protease. In addition, the production of novel enzymes through protein engineering technologies such as directed evolution, rational, and de novo design, for the improvement of existing industrial enzymes is also explored. We have also explored the role of metagenomics, nanotechnology, OMICs, and machine learning approaches in the bioprospecting of novel enzymes. Overall, this review covers the basics of biocatalysts in industrial and healthcare applications and provides an overview of existing microbial enzyme optimization tools.

Key points

- *Microbial bioactive molecules are vital for therapeutic and industrial applications.*
- *High-throughput OMIC is the most proficient approach for novel enzyme discovery.*
- *Comprehensive databases and efficient machine learning models are the need of the hour to fast forward de novo enzyme design and discovery.*

Keywords Industrial enzymes · Microbial enzymes · Bio-prospection · Industrial biotechnology · Enzyme design

Introduction

Biocatalysts are enzymes having high specificity and activity against targeted substrate that promotes bioconversion rate (Robinson 2015; Jemli et al. 2016). Metabolic pathways in the living cell depend on multiple enzymes for breaking down macromolecules into simpler units that are easier to absorb by the cells (Jemli et al. 2016; Piergiorge et al. 2017; Hughes and Lewis 2018). Such enzymes could be exploited to catalyze a vast array of commercial processes in the textile, dairy, and pharmaceutical industries (Morin et al. 2019). Applications of microbial enzymes date back thousands of years when the Sumerians fermented brew around 7000 years ago and utilized chymosin, a protease from ruminant animals. However, modern biocatalysis was first established by Louis Pasteur in 1876, followed by the postulation of the "lock and key" model by Emil Fisher in 1894 that demonstrated the enzyme specificity. In 1897, Buchner proposed that the fermentation process be catalyzed with cell-free extracts; this paved the beginning of enzyme-catalyzed fermentation processes (Grunwald 2017; Hughes and Lewis 2018). A schematic diagram of the historic millstones in the application of biocatalysts is depicted in Fig. 1. The usage of industrial enzymes has been increasing rapidly due to improved performance and cost-effectiveness. The ever-increasing demand could be attributed to the stability in a range of substrate specificity, pH, temperature, and biodegradability (Jemli et al. 2016; Grunwald 2017). By the beginning of the twenty-first century, the enzyme industry had a remarkable growth and expansion that provided jobs, goods, services, and opportunities to improve the living standard for millions of people globally.

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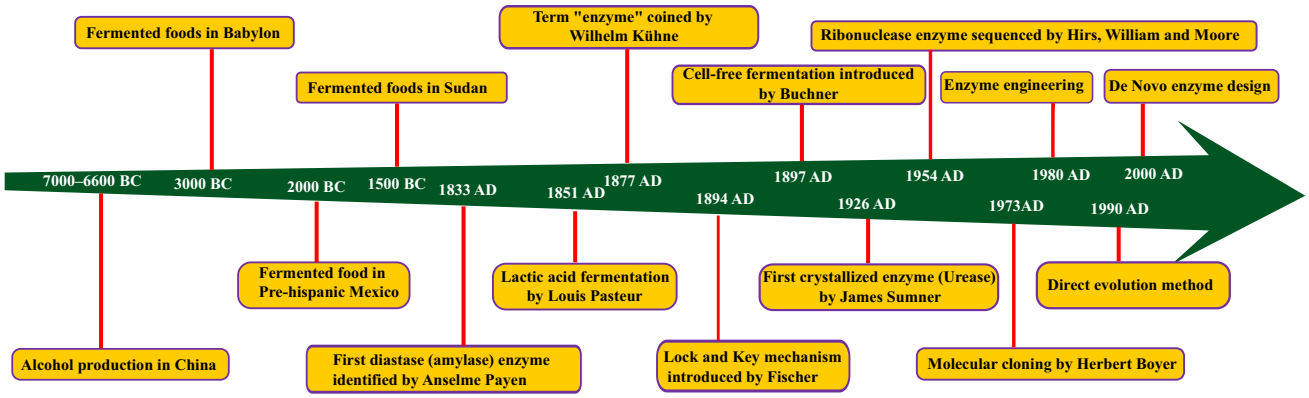

Fig. 1 Schematic representation of enzymatic usages and discoveries in chronological order

Most of the industrial enzymes are of animal sources (chymosin, pepsin, trypsin, and pancreatin), plant sources (ficin, papain, and bromelain), and microbial sources (pectinases, glucoamylase, α-amylase) (Tan et al. 2019; Daniell et al. 2019; Saad et al. 2020). However, in particular, microbial enzymes have become an invaluable part of pharmaceutical, food, detergent, leather, textile, paper, biofuels, food, beverage, and other consumer products (Morin et al. 2019). The importance of microbial enzymes has been credited to their higher stability and activity compared to plant and animal sources (Gurung et al. 2013). In addition, microbial enzymes can be produced in a larger quantity through fermentation processes in minimum time. Hence, industries have focused on identifying novel strains to produce specific enzymes of desirable properties (Anbu et al. 2017). Furthermore, with the advancement of synthetic biology and tweaking enzyme structure(s), microbial enzymes have provided a promising future with customized functions that have attracted researchers from academia and industries (Rao et al. 2017). In this review, we have explored the common industrial enzymes, their classifications, and applications. Furthermore, we also explored conventional approaches for enzyme bioprospection and design such as rational, de novo, and directed evolution, followed by the upcoming tools such as metagenomics, OMICs, nanotechnology, and machine learning that have reshaped the enzyme discovery processes.

Industrial applications of microbial biocatalysts

Microbial enzymes have been an essential tool in the food, agricultural, pharmaceutical, and cosmetics industries. Rapid discoveries of unique stable microbial enzymes in extreme conditions that are cost-effective, ease in scaling up, manipulation, and optimization have made the application of microbial enzymes an indispensable biological tool in various industries (Gurung et al. 2013; Moopantakath and Kumavath 2018). Carbohydrase are enzymes that catalysis carbohydrates, and their products are used as raw materials in industries (Cole et al. 2019). The application of carbohydrates in food industries includes baking, starch liquefaction, brewing, clarification of fruit juice, and therapeutics such as dextrin, heparin, and hyaluronan (Kolb and Sharpless 2003). The list of standard industrial enzymes and their functions are listed in Table 1 and Fig. 2. Lipases are crucial enzymes found in all organisms that hydrolysis triglycerides. In humans, they hydrolysis fatty acids and lipids in the pancreas and stomach. Microbial lipases have an essential role in biofuel, food, animal feed, dairy, etc. The global lipase market consists of ~ 90% microbial lipases (Raveendran et al. 2018). Microbial enzymes such as xylanases are vital in hemicellulose degradation for biofuel production. Hemicellulose is one of the most abundant polymers and consists of xylan, xyloglucan, glucomannan, galactoglucomannan, and arabinogalactan (Collins et al. 2005). The hemicellulose degradation requires multiple enzymes such as endoxylanases, β-xylosidases, feruloyl esterase, etc. Xylan is the major component of hemicellulose, and xylanases that break down xylan are important in the industrial sector, particularly biofuel. Similarly, inulinases are hydrolases that hydrolysis the β-2,1-glycosidic bonds that links fructose residues in inulin. Hydrolysis of inulin yields fructose and glucose with industrial and medical importance (Singh et al. 2007). Since inulins are the major carbohydrate reserve in plants, the dried biomass in tubers comprises up to 70% inulin (Chi et al. 2009). The abundance of inulin makes the application of inulinases attractive (Singh and Singh 2016). The list of enzymes with medical and industrial significance is prohibitively lengthy to discuss in detail in this chapter. Hence, this section describes the recent advancements and applications of cellulase, pectinases, amylase, and proteases.

Table 1 List of enzymes and their sources, functions, and applications in industry

Name of the enzymes with EC code	Sources	Enzyme functions	Industrial applications	References
Endopolygalacturonase (EC 3.2.1.15)	*Fusarium oxysporum f.* sp. *Lycopersici Aspergillus aculeatus Rhizoctonia fragariae Peacilomyces clavisporus* 2A *Saccharomyces cerevisiae*	Hydrolyzes alpha-1,4 glycosidic bonds between galacturonic acid residues	Textile, wood and food industries Ripening agent Wine preparation Processing of animal feed Tea and coffee processing Act as bioleaching agent Extraction of vegetable oil Recycling of wastepaper	Cho et al. 2001 Souza et al. 2003 Kluskens et al. 2005 Evangelista et al. 2018 Bonfá et al. 2018 Liu et al. 2018 Chen et al. 2018 Mohandas et al. 2018
Exopolygalacturonase (EC 3.2.1.67)	*Bifidobacterium longum Bacillus velezensis Myceliophthora thermophila Bacillus* sp.	Hydrolyze non-reducing end of the polymer a monosaccharide galacturonic acid		
Pectate lyase (EC 4.2.2.2)	*Bifidobacterium longum Bacillus velezensis Bacillus sonorensis* MPTD1	To cleavage of (1→4)-α-D-galacturonan to give oligosaccharides with 4-deoxy-α-D-galact-4-enuronosyl groups		
Pectin esterase (EC 3.1.1.11)	*Bacillus sonorensis* MPTD1	Deesterification of pectin methoxyl group		
Pectinase (EC 3.2.1.15)	*Fusarium* sp. *Rhizoctonia fragariae Paecilomyces variotii Bacillus* sp. *Erwinia carotovora Bacillus subtillis Aspergillus niger* IM-6 *Aspergillus aculeatus Erwinia carotovora Halobacillus Thalassobacillus Halomonas Salicola Bacillus* sp. *MFW7 Bacillus sonorensis MPTD1 Bacillus sonorensis Clostridium* sp. *Aspergillus* sp. *Penicillium* sp.	Degrade pectin	Textile industry Bioscouring ability Paper and pulp industry Food processing industry Wastewater treatment	Akhter et al. 2011 Nisha 2016 Mohandas et al. 2018 Rajendran et al. 2011 Chiliveri et al. 2016 Ahlawat et al. 2007 Schols et al. 1990
Cellulase (EC 3.2.1.4)	*Bacillus subtilis* IARI-SP-1 *B. amyloliquefaciens* IARI-SP-2 *Humicol ainsolens Trichderma reesei Trichoderma Bacillus subtilis* BL62 *Aspergillus niger T. viride T. harzian Humicola Trichoderma reesei Chaetomium Trichoderma Fusarium Penicillium Aspergillus Actinomycetes Ruminococcus albus Cellulomonas* spp. *Pseudomonas* spp. *Escherichia coli Bacillus* spp. *Serratia marscens Humicola insolens Bacillus* sp.	Degradation of cellulose	Pulp and paper industry Textile industry Food processing industry Detergent industry Carotenoid extraction Agricultural industries	Mitchinson and Wendt 2001 Pandey et al. 2014 Bailey and Lumsden 1998 Harman and Kubicek 1998 Abdul et al. 2015 Nam et al. 2016 Sethi et al. 2013 Galante et al. 2014 Schmoll and Kubicek 2003 Heck et al. 2002

Table 1 (continued)

Name of the enzymes with EC code	Sources	Enzyme functions	Industrial applications	References
Amylase (EC 3.2.1.1)	*Fungal amylase B. amyloliquefaciens* EMS-6 *Bacillus stearothermophilus Bacillus Aspergillus* sp. *Aspergillus fumigatus Haloferax mediterranei Halobacillus* sp. *Ferdowsicous* sp. *Bacillus* sp. *strain SMIA-2 Bacillus* sp. *Ferdowsicous*	Cleaves 1,4-α-D-glycosidic bonds and convert into glucose monomers	Food, detergents, feed, leather, textile, paper, and pulp industry Fast and secure weaving via improving strengthen agent Modified retrogradation in starch hydrolysis Production of single glucose units from the amylose and amylopectin	Saxena et al. 2003 Mitidieri et al. 2006 Singh et al. 2014 Haq et al. 2010 Souza et al. 2015 Arshad et al. 2016 Menasria et al. 2018 Cordeiro et al. 2002 Peng et al. 2014 Mitrofanova et al. 2017 Iwase et al. 2010 Longhi et al. 2008 Elchinger et al. 2014 Neelakantan et al. 1999 Asoodeh et al. 2010
Proteases (EC 3.4)	*Penicillium Rhizopus Bacillus* sp. *Mucor* sp. *Bacillus subtilis Lactobacillus acidophillus Aspergillus oryzae Endothia parasitica Irpex lactis Bacillus pumilus S. epidermidis Serratiopeptidase Bacillus amyloliquefaciens Bacillus licheniformis*	Hydrolyze peptide bonds of proteins	Detergent and pharmaceutical production of peptides and amino acids from proteins	
Lipase A (EC 3.1.1.3)	*Bacillus* sp. *Pseudomonas* sp. *Rhizopus* sp.	Hydrolysis of long-chain triglycerides	Detergent, pulp, and paper	Hasan et al. 2006
Inulinase (EC 3.2.1.7)	*Aspergillus niger*	The β-2, 1 linkage of fructopolymers	Food industry	Dinarvand et al. 2012
Xylanase (EC 3.2.1.8)	*Thermomono spora fusca*	Hydrolysis of xylan	Kraft pulps	Goswani et al. 2015

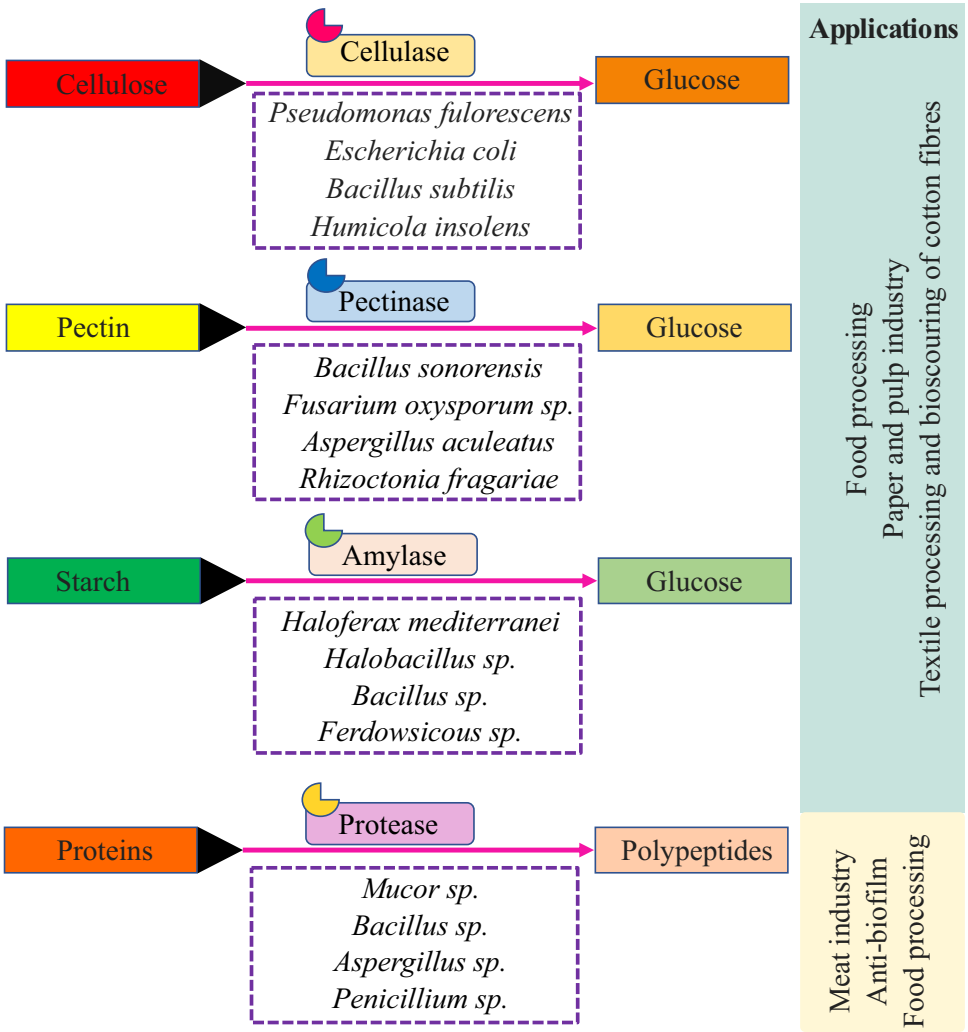

Fig. 2 Industrially important microbial enzymes are produced by several species with varying properties. Some of the sources of enzymes responsible for the degradation of cellulose, pectin, starch, and proteins and their application are depicted in the figure

Cellulase

Cellulose is a polysaccharide containing linear D-glucose chains linked with β-1,4-glycosidic bonds and insoluble in various solvent systems, including water. Cellulose-degrading enzymes, known as cellulase, are highly abundant in all domains of life (Bonfá et al. 2018). Cellulase is classified into endocellulase, exocellulase, and β-glucosidases (Bergmann et al. 2014; Cunha et al. 2016). Endocellulase acts on the non-covalent bonds in cellulose to produce new chain ends. These chain terminals are broken down into smaller sugar units by exocellulase. The mixture of endocellulase, exocellulase, and cellobiases is commercially available in the market as cellulase (Bonfá et al. 2018; Barkalow and Whistler 2014). Cellulase-producing microbes such as *Cellulomonas* sp., *Pseudomonas* sp., *Escherichia coli*, *Bacillus* sp., and *Serratia marscens* are mostly isolated from alkali soil. Cellulase applications include cotton softening, food mashing, wastewater treatment, and pharmaceuticals (Sethi et al. 2013).

Applications of cellulase

Mechanical pulping has been the primary choice for grinding and refining rigid wood materials in the paper making process, although it is uneconomical. Application of cellulase enzymes reduces up to 40% of production cost with no adverse effects. Its role includes reducing pulp viscosity, deinking paper waste, and preventing alkaline yellowing by applying acidophilic cellulase (Mai et al. 2004; Singh et al. 2007; Rathnan and John 2020). In addition, endoglucanase isolated from *Humicola insolens* and β-1,4-endoglucanases from *Bacillus* sp. enhances the paper pulp treatment and improves drainage properties of the pulp. Hence, they are broadly used to manufacture biodegradable and

environmentally friendly products such as cardboard towels and sanitary paper (Pandey et al. 2014; Abdul et al. 2015).

Textile industry Cellulase plays an imperative role in the textile industry by improving the appearance, desizing, and dye adherence to the fabrics (Kuhad et al. 2011). Cellulase also acts as an excellent finishing agent by enhancing the softness, water absorbance, and a clean, shiny look to the textile products. Endoglucanase II, produced by *Trichoderma reesei*, is widely used as a stone washing agent in American laundry industries (Galante et al. 2014).

Food processing industry Cellulase helps retain and maintain the flavour, texture, and aroma of vegetables and fruits. Cellulase is used as a macerating agent for decreasing viscosity and bitterness. It is also combined with other macerating enzymes to extract olive oils (De Faveri et al. 2008; Singh et al. 2019a). For instance, the combined action of cellulase, pectinase, and xylanase provide better extraction, filtration, and reduced viscosity in the wine industry (de Carvalho et al. 2008; Bajaj and Mahajan 2019). In addition, microbial cellulase such as those derived from *Trichoderma* species has been found to enhance feed conversion and increase cereal-based food digestibility (Schmoll and Kubicek 2003). For example, cellulase from *Bacillus subtilis* BL62, isolated from soybean residue, showed cellulase activity of 1.08 UI/mg proteins within 24-h incubation (Heck et al. 2002).

Agricultural industries Cellulase enzymes play an essential part in the agricultural sector for seed germination, improved root system, flowering, increased crop yield, and defense against phytopathogens (Bhat 2000). Cellulase also improves soil fertility; hence, microbes such as *Aspergillus*, *Chaetomium*, and *Trichoderma* helps to increase soil fertility that promotes plant growth. Fungal cellulase can be isolated from several fungi such as *Fusarium*, *Penicillium*, *Aspergillus*, and bacterial cellulase from *Actinomycetes* and *Ruminococcus albus* (Bailey and Lumsden 1998; Harman and Kubicek 1998).

Pectinases

Pectins are the crucial ingredient in the middle lamella of the plant cell wall. They are subsequently modified and remodelled during plant growth and development. Pectins have an essential role in nutrient regulation, signalling, and defensive mechanism (Ridley et al. 2001; Wyman et al. 2005). Extraction of pectin and its derivatives through chemical methods such as acid extraction, precipitation, and drying is commonly practiced. However, downstream purification processes are complicated and expensive. Pectinase from microbial origin has high efficiency and minimal purification requirements than plant sources.

Pectinases are classified as pectinesterase, depolymerizing enzymes (pectinase), and protopectinase. Pectinase found in *Bacillus sonorensis* MPTD1 strain esterifies the pectin's methoxyl group (Mohandas et al. 2018). Endopolygalacturonase (EC 3.2.1.15) and Exopolygalacturonase (EC 3.2.1.67) are used in the food industry to produce short pectin molecules by hydrolyzing internal and external glycosidic bonds, respectively. This increases the yield of juices, decreases viscosity, and determines the product crystallinity. Endopolygalacturonase (EC 3.2.1.15) hydrolyzes the galacturonic acid residues in its α-1,4-glycosidic bonds (Tapre and Jain 2014). Some of the microbial sources of Endopolygalacturonase include *Fusarium oxysporum* sp., *Lycopersici*, *Aspergillus aculeatus*, *Rhizoctonia fragariae*, *Peacilomyces clavisporus* 2A, and *Saccharomyces cerevisiae* (Cho et al. 2001; Souza et al. 2003). Exopolygalacturonase (EC 3.2.1.67) can be extracted from *Bifidobacterium longum*, *Bacillus velezensis*, and *Myceliophthora thermophila Bacillus* sp. (Liu et al. 2018; Chen et al. 2018). They produce galacturonic monosaccharide acid by hydrolyzing the non-reducing end of the polymer.

Applications of pectinase Pectinase is a dominant (25%) part of the enzyme market due to its multidisciplinary application such as vegetable oil extraction, fruit, wine, tea, coffee, and animal feed processing (Prathyusha and Suneetha 2011). It also acts as a bioleaching agent and helps recycle wastepaper (Pivnenko et al. 2015). In addition, pectinase regulates viscosity, clearing up, color liberation, and enhancing the overall yield of food products. Microbial pectinase is diverse, especially in phytopathogen such as *Halobacillus*, *Thalassobacillus*, *Halomonas*, *Salicola*, *Bacillus* sp. MFW7, and *Bacillus sonorensis* MPTD1 (Mohandas et al. 2018).

Textile processing and bioscouring of cotton fibers Textile industries constantly need sustainable and eco-friendly fabric processing. Caustic soda was used as a sizing agent. However, its toxic and damaging effects on the fabric make it highly undesirable. Therefore, the industry requires enzymes that are stable in a harsh environment yet safe and cost-effective. It has raised the need for novel pectinase for scouring (Jayani et al. 2005). Bio scouring is the application of pectinase to remove pectins and waxes from fabrics under alkaline conditions. Pectinase enzymes isolated from the *Fusarium* sp. and *Paecilomyces variotii* have strong bioscouring ability, including water absorption and tensile strength compared with conventional methods (Rajendran et al. 2011; Nisha 2016).

Paper and pulp industry Pectinase is preferred because conventional methods use alkaline peroxide, affecting the

reaction and end product. Hence, microbial pectinase isolated from *Bacillus* sp. and *Erwinia carotovora* is used in paper preparation. Pectinases from bacteria (e.g., *Clostridium*, *Bacillus*) and fungi (e.g., *Aspergillus*, *Penicillium*) are also widely used for the retting process, which involves fermentation of bark and pectin to release fibers (Chiliveri et al. 2016). Heat-resistant pectinases from *Bacillus subtillis* increase the pulp whiteness and reduce alkali consumption and production costs (Ahlawat et al. 2007).

Food processing industry In commercial food industries, extraction of cleared juice products without turbidity is desirable. Conventional methods have a higher chance of causing contamination and deformed maturity due to released heat. Pectinase breaks down pectin into negatively charged galacturonic acid, forming a complex with positively charged protein, resulting in a clear juice (Hmid et al. 2016). *Aspergillus aculeatus* produce rhamnogalacturonase that can be used to macerate apples (Schols et al. 1990). Pectinase, particularly of fungal source, is commonly used in the coffee industry to remove mucilage coat. Pectinase from fungal species *Aspergillus niger* IM-6 exhibits high pectinase activity against wheat bran (Akhter et al. 2011; Alazi et al. 2018).

Amylase

Raw materials such as plant seeds, roots, and tubers are a rich starch sources, and their derivatives have a complex structure. Starch is a polysaccharide constituted by glucose chains connected with a glycosidic bond (Li et al. 2021). Amylase breaks the glycosidic bonds and produces glucose monomers. Amylases are used to prepare bread, detergent, textile desizing, paper industry, and biofuel production (Kumar et al. 2014). Microbial amylases are in high demand due to their tolerance to multiple conditions such as salt, temperature, pH, and different stress conditions (El-Fallal et al. 2012). Three major groups of amylases have been classified based on their chemical nature, α-amylase, β-amylase, and γ-amylase.

α-Amylase (EC 3.2.1.1) α-Amylase is a digestive enzyme responsible for α bond hydrolysis in the α-linked polysaccharide. They are in high demand for commercial processing of glucose and maltose. α-Amylase is the largest of amylase across all taxonomic kingdoms. Salt-loving α-amylase has been isolated from haloarchaea *Haloferax mediterranei*, which habitats high saline ecosystems (Perez et al. 2003; Menasria et al. 2018). Thermophilic amylase has been reported in *Bacillus sp*. strain SMIA-2, while acidophilic amylase was isolated from *Bacillus sp. Ferdowsicous* (Cordeiro et al. 2002; Asoodeh et al. 2010). α-Amylase is used in the fruit juice industry, wastewater treatments, bioethanol production, and textile industry (Dey and Banerjee 2014; Kumar et al. 2014; Pervez et al. 2014; Raul et al. 2014).

β-Amylase (EC 3.2.1.2) The second-largest amylase group is β-amylase, producing maltose as a hydrolysis product. There are various β-amylase found in bacteria, fungi, archaea, and plants. For example, β-amylase from salt-loving species *Halobacillus* sp. can tolerate high saline stress conditions (Peng et al. 2014).

γ-Amylase (EC 3.2.1.3) The third type of amylase group is γ-amylase that acts on amylose and amylopectin either at α(1–6) and last α(1–4) glycosidic linkage and produces glucose. The unique characteristic of γ-amylase is its resistance in acidic media (Sivaramakrishnan et al. 2006; Hiteshi and Gupta 2014).

Applications of amylase Amylase helps in coating paper with starch compounds of high molecular weight and low viscosity. Coating treatments with amylase enhances the writing quality and smoothness of paper. Common commercial amylase used in the paper industry is Amizyme® and Fungamyl BAN® (Saxena et al. 2003).

Textile processing and bioscouring of cotton fibers Amylase is used to fast, secure desizing activity, and improve weaving. Amylase isolated from *Bacillus* has indispensable application in the textile industry. Mutant strains are prepared by treating wild strains with ethyl methanesulphonate. *Bacillus amyloliquefaciens* EMS-6 had a 1.4-fold higher α-amylase production than the wild type (Haq et al. 2010).

Food industry Amylase has a wide application in food industries such as fruit juice processing, brewing, cakes production, and baking for reducing dough viscosity and improving quality such as taste, color, and quantity. Starch modification activity by amylase from *Bacillus* sp. increased with temperature (Souza et al. 2015). About 90% of all detergent contains amylase enzymes to remove stains and dirt from the fabrics or cloth without altering the maturity. Amylase can also act in lower temperatures and alkaline conditions. As a result, whiteness in the fabrics is increased by decreasing the adhesion of the starch compound. Amylase isolated from *Bacillus* or *Aspergillus* has high detergent activity (Mitidieri et al. 2006; Souza et al. 2015).

Proteases

Protease is a naturally occurring enzyme found in most living cells performing vital physiological functions such as post-translational modification, inflammation, zymogen

activation, and blood coagulation (Martin et al. 2021). They have a central function in nitrogen metabolism and absorption of amino acids into cells through protein hydrolysis (Sabotic and Kos 2012; Souza et al. 2015). They are the single most prominent gene family making up approximately 2% of our genome (Li et al. 2013). However, most protease initially produced in nature are inactive precursors called zymogens or proenzymes to prevent unwanted proteolysis. To obtain the active form, they undergo environmentally induced conformational changes or cleavage of the activation segment (Li et al. 2013).

Proteases belonging to the hydrolase class (EC.3) are grouped under the peptide hydrolases or peptidases subclass (EC 3.4). Proteases that act on the N- or C-terminal of the substrate is known as exopeptidases (EC 3.4.11–19), and those that act within the substrate are known as endopeptidases (EC 3.4.21–99). The exopeptidases are further subcategorized as aminopeptidases (EC 3.4.14) that act on the N terminus or carboxypeptidases that acts on the C terminus (Motyan et al. 2013). Like other enzymes, protease is also pH-dependent. Hence, protease is also categorized according to their optimum pH, such as acidic proteases (pH < 7.0), neutral (around pH 7.0), or alkaline (pH > 7.0) (Gupta and Ayyachamy 2012; Tavano et al. 2018).

Applications of protease Microbial protease has applications in the food, dairy, and textile industries. Protease is the most in-demand industrial enzyme and covers up to 65% of the global enzyme market (Pant et al. 2015). They have a wide array of applications ranging from detergents to pharmaceuticals. They are used in food, dairy, textile, and contact lens cleaner. In academia, they have wide applications such as peptide synthesis, DNA extraction (digestion of unwanted proteins), tissue culture (tissue disassociation), and removal of affinity tags (expression cloning) (Motyan et al. 2013). One of the primary producers of proteases in the industry is *Bacillus subtilis* due to its wide spectrum of properties.

Protease production is preferred from microbial origin due to its ease in production processes. Most microbial proteases are extracellular and secreted into the fermentation medium, making it easier for downstream processing (Savitha et al. 2011). In addition, fungal protease production has also been highlighted in the past due to its relatively low media cost, faster production, and ease of separation of mycelium by filtration (Anitha and Palanivelu 2013; Souza et al. 2015). However, enzymes produced should be safe, including the microorganism producing it. Hence, such safety measures are checked and certified by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (Tavano et al. 2018). One of the significant advantages of fungi mediated protease production is its safety tag since they are mostly considered GRAS (generally regarded as safe) (Germano et al. 2003; Souza et al. 2015). Some of the fungal protease producers used in industry are *Aspergillus* species, *Penicillium* species, *Mucor pusillus*, and *Mucor miehei* (Souza et al. 2015).

Meat industry In the meat industry, one of the factors that determine meat quality is meat tenderness. The toughness of meat is depended on several factors, including pH, temperature, muscle type, and age of the meat source (Anderson et al. 2012; Arshad et al. 2016). Proteases are used to break down the peptide bonds in muscles and hydrolyze components of connective tissue fibres such as collagen and elastin. For this purpose, proteases from fungal and bacterial sources, such as *Aspergillus oryzae* and *Bacillus subtilis*, are widely used as the US Department of Agriculture (Arshad et al. 2016) approves them.

Dairy food processing Food products contain complex constituents, including proteins and peptides that interact to get unique features of each food (Tavano et al. 2018). Protease treatment of food products can enhance the texture, flavor, aroma, and digestibility (Lacou et al. 2015). In cheese making, a high specific protease is used to disturb the casein micelles. Traditionally, this is achieved by applying chymosin protease from calf rennet which hydrolysis the peptide bond in Phe105, Met106 of kappa casein to form para-casein and macro peptides (Tavano et al. 2018). It destabilizes the casein micelles and aggregates to form milk coagulation. The product is precipitated and form gel-like properties, further developing into a continuous chain of 3D structure. Microbial cultures can be used as starter cultures to speed up the cheese ripening process. Several proteases of microbial origin can perform similar actions targeting different peptides. This gives the option to produce cheese of different quality, texture, and aroma. Several microbial species are used for protease production, such as *Lactobacillus acidophillus*, *Aspergillus oryzae*, *Endothia parasitica*, *Irpex lactis*, etc. (Neelakantan et al. 1999).

Anti-biofilm Several pathogenic bacteria form biofilm for protection against drugs (Sharma et al. 2019). The protective biofilm coating nullifies the effectiveness of drugs on such pathogens. Although biofilm formation varies substantially between bacterial types, there are specific common properties involved in all of them, such as forming a thick extracellular matrix accounting for over 90% of the biomass, attachment to the surface, and aggregation of cells (Mitrofanova et al. 2017). Biofilm formation starts with the initial attachment of the bacteria to the surface through autolysin and adhesion, which becomes irreversibly attached and accumulates further in several layers (Rao and Kumavath 2020). The cells are released into the surrounding environment upon maturation, leading to further biofilm formation

(Elchinger et al. 2014). Current antibiotics could inhibit such pathogens if the biofilm is destructed. For example, *Serratia marcescens* extracellular biofilm matrix was effectively degraded using subtilisin-like protease and glutamyl endopeptidase from *Bacillus pumilus* (Mitrofanova et al. 2017). Microbial protease such as serine protease from *S. epidermidis* and *serratiopeptidase* from *S. marcescens* has also been an effective biofilm suppressor against *S. aureus* and *Listeria monocytogenes*, respectively (Longhi et al. 2008; Iwase et al. 2010). In addition, protease enzymes of other classifications such as flavourzyme (from fungal *Aspergillus oryzae*), neutrase (from *Bacillus amyloliquefaciens* under Firmicutes phylum), and serine endopeptidase alcalase (from *Bacillus licheniformis* under Firmicutes phylum) has been shown to exhibit anti-biofilm formation through exolytic and endocytic mechanisms (Elchinger et al. 2014).

Resources and prospects of novel enzymes

Although several thousands of microbial enzymes have been identified with bioactivity, the enzyme market reports only about 200 enzymes of microbial origin (Ranjith et al. 2008; Li et al. 2012a, b and Kumavath et al. 2015). The market is highly competitive, and the profit margin is minimal; hence, dominated by a few companies such as Novozymes, DuPont, Roche, Amway, and BASF. Bioprospection of novel enzymes has been extensively carried out in terrestrial ecosystems due to its ease of accessibility for sampling. In the recent decade, extremophiles from extreme environments such as saltern, hot springs, Arctic sea, etc., have also been harnessed for novel enzymes. The marine ecosystem is a massive reservoir of enzymes and has gained interest in recent years (Zhang and Kim 2010; Rao et al. 2017). However, the vast marine ecosystem is still under-sampled. Besides, marine ecosystems such as mangroves forest sediment harbour a considerable diversity of microbes having unique biological functions. The variety of microbial communities in marine environments such as mangrove sediments differs worldwide, raising the odds for novel enzyme discoveries (Imchen et al. 2017).

Metagenomic approaches for novel enzyme discovery

Enrichment of environmental/biological samples in selective media for pure culture isolation and enzyme characterization has been the primary approach for novel enzyme bioprospection. However, most enzymes are re-discovered through the culturable process due to the narrow spectrum of culturable microbes. It is a severe setback because more than 98% of the microbes are uncultivable under standard laboratory conditions and growth media (Garrido et al. 2017). A recent approach to tackle the issues is the application of metagenomic that can address the challenges in two broad ways: sequence and expression-based metagenomics. In sequence-based metagenomic, novel enzymes are mined based on protein homologs of novel sequences against a database of already known enzymes. Several gigabytes of metagenomic data are screened for proteins having identical homologous sequences in this technique. Such sequences are synthesized and inspected to express in a suitable vector-host system for desired activity. In expression-based metagenomic, random metagenomic DNA fragments of ~ 300 bp to several kilobases are ligated onto an appropriate vector, which is then screened for expression against the desired substrate or phenotypic changes. Using functional metagenomics, novel enzymes that could not have been predicted with sequence-based techniques have been discovered, such as novel β-galactosidases and novel esterase of GDSGG motif (Cheng et al. 2017; Jayanath et al. 2018). An amalgamation of sequence and expression-based metagenomic is known as screening gene-specific amplicon from metagenomes (S-GAM). A primer is designed to amplify the full-length gene of interest within a metagenome; the amplicon is subsequently cloned and screened for expression. Alcohol dehydrogenase (ADH) and phenyl acetaldehyde reductases have been discovered using the S-GAM technique (Itoh et al. 2014 and 2016).

The mining of novel enzymes can be tedious due to the exponential increases of metagenomics data and the computational power requirements. Therefore, Anastasia was developed as a Web server application (Koutsandreas et al. 2019). Anastasia is an acronym for "automated nucleotide amino acid sequences translational platform for systemic interpretation and analysis." It has been used extensively for the mining of enzymes from thermal springs whole over the world to find industrially interested enzymes. For example, the Anastasia platform discovered a novel thermostable esterase EstDZ4 from Krisuvik, Iceland (Koutsandreas et al. 2019) (Table 2).

Strategies for novel enzyme development

Genetic engineering has opened up the possibility of developing enzymes of desired properties with low production costs (Jemli et al. 2016). The enzymes can be engineered to operate on a range of substrates and concentrations, extremes of temperature, pH, pressure, and stability in non-aqueous solvents to facilitate substrate solubility and product extraction (Lin and Tao 2017). Naturally, discovered enzymes do not possess such attributes since they are adapted to the natural environment. Hence, natural enzymes would have the optimum functions at

Table 2 List of modern omics tool for the enzymatic research

Omics methods	Tools	Enzymes discovered	Reference
Genomics	Next-generation sequencing	Proteases, cellulase, β-glycosidase, pectinase, Tannas, di-chlorophenol hydroxylase	Sun et al. 2020 Chai et al. 2020 Bergmann et al. 2014 Singh et al. 2012 Yao et al. 2011 Lu et al. 2011
Transcriptomics	RNA-sequencing	Cellulase, xylanase, pectinase	Yan et al. 2017 Alazi et al. 2018
Proteomics	LC–MS, MALDI	Keratinase, γ-glutamyltranspeptidase, chitosanases, glicosidases	Parrado et al. 2014 Kirkland et al. 2008
Metabolomics	LC–MS, GC–MS, NMR	Cellulase, glycolytic enzymes	Vanee et al. 2017 Prosser et al. 2014 Ming et al. 2018 Amer and Baidoo 2021

the physiochemical condition observed in nature. Therefore, modification of the enzyme is required to provide the necessary stability and activity (Milner and Maguire 2012). Protein engineering offers exceptional prospects for designing industrial enzymes at lower production costs (Jemli et al. 2016).

Directed evolution Directed evolution commences with a parent protein and aims to enhance selectivity or protein stability on a particular substrate. Directed evolution for enzyme engineering consists of two main steps, i.e., generating a library of gene variants through random mutagenesis or recombination, followed by screening the variants with the preferred function (Arnold and Volkov 1999; Arnold and Georgiou 2003; Castle et al. 2004). The resultant gene can be cloned into a suitable host to prepare starter culture, which can be used as a biocatalyst in industrial processes (MacBeath et al. 1998). Practically, repeated rounds of mutations and selection have to be carried out to improve protein stability and enzymatic function (Floor et al. 2014). The best performing variant can be selected as the template in each cycle. The development of hyperactive biocatalysts reduces the production costs of industrial goods and reduces industrial waste (Hughes and Lewis 2018; Qu et al. 2020). Directed evolution of natural or repurposed enzymes is based mainly on the rapid and high throughput library creation and screening of the desirable products (Turner 2009). A new technique for mutation library creatures is the on-chip solid-phase gene synthesis (Packer and Liu 2015).

Directed evolution can also be based on the shuffling of genes homologues to the gene of interest. One of the strengths in directed evolution, compared to rational design, is that prior knowledge of the structural information is not required (Waldo 2003). This is particularly important for novel natural enzymes (Table 3) without experimentally elucidated structures. Modifying natural enzymes is necessary since most enzymes do not function at their best efficiency outside of their natural microenvironment. Hence, modification of the enzymes through directed evolution is one of the primary approaches to obtain improved traits such as selectivity, stability, and activity (Zeymer and Hilvert 2018).

Rational design Rational protein engineering is a technique where enzymes are engineered with enhanced desired features from an existing enzyme without losing the original properties, including its stability, specificity, and activity (Milner and Maguire 2012; Dinmukhamed et al. 2021). In rational design, protein structures are initially analyzed in silico (Fig. 3) for possible modifications to enhance the desired features (Ding and Dokholyan 2006), followed by in vitro gene alteration through site-directed mutagenesis (SDM) and transforming into a suitable expression host, e.g., *E. coli* (Bornscheuer and Pohl 2001). The insert is expressed and purified to analyze the desired properties (Hart and Tarendeau 2006; Michael et al. 2015). Based on the target sites, SDM of the candidate residues is performed to generate multiple libraries of mutant strains. The strains are screened for selection of desired functionally enhanced variant.

The first step in the rational design of enzymes is the multiple sequence alignment (MSA) of the candidate sequence against a reference database to identify potential sites involved in substrate specificity and functional site residue that can be mutated for variant library generation (Steiner and Schwab 2012). A standard approach to perform MSA for highly similar homologous sequences is through the progressive alignment algorithm ClustalW (Reddy and Fields 2022). The alignment data serves as the raw information for co-evolutionary analysis based on the evolutionary substitution of the amino acid residues under evolutionary pressure (Modi et al. 2021; Frappier and Keating 2021). Co-evolutionary analysis helps identify functional sites and protein structures that can assist in the customization of putative functional sites. Several software implements multiple algorithms for the co-evolutionary analysis. For instance, OMESKASS is software that implements Observed Minus Expected Squared (OMES) for co-evolutionary analysis. Similarly, the Fodor package was recently developed that have several algorithms, including mutual information (MI) (Kuhlman and Bradley 2019), McLachlan-based substitution correlation (McBASC), and statistical coupling analysis (SCA). Among those algorithms, it has been reported that McBASC (Swint et al. 2021) and OMES have better

Table 3 The design strategies and methods used to optimization targeted enzymes

Enzyme name	Optimization target	Method and design strategy	Function	References
Xylanase	pH optium	Rational design	Paper and pulp industry pKaf prediction	Pokhrel et al. 2013
Phosphoglycerate dehydrogenase	Allosteric sites	Rational design	Inhibits the synthesis of serine and Glycine in cancer cells	Wang et al. 2017
Amine oxidase	Substrate range	Rational design	Medical application	Ghislieri et al. 2013
Carboxylesterase 1	Fluorescent Probe	Rational design	Optical properties	Tian et al. 2019
2-Phenylcyclopropane carboxylic acids		Rational design	inhibiting O-acetylserine sulfydrylase	Pieroni et al. 2016
Dipeptidyl Peptidase 4	Pharmacokinetics	Rational design	Treatment of type 2 diabetes	Li et al. 2016
Tipranavir	Non-peptidic activity	Rational design	Antiviral activity	Doyon et al. 2005
Xylose dehydrogenase	Thermostability	Rational design	Applied to construct xylose/O_2-based biofuel cell	Feng et al. 2016
Orthogonal aminoacyl-tRNA synthetases	Enzymatic efficiency	Directed evolution	Enables site-specific installation	Bryson et al. 2017
Oxygenases	Hydroxylation optimizing	Directed evolution	To generate fluorescent compounds	Joo et al. 1999
Cytochrome P450	Activities substantially	Directed evolution	Nitrene transfer reactions	Roiban and Reetz et al. 2015
Pectate lyase	Thermostable	Directed evolution	Cotton fabric	Solbak et al. 2005
Haloalkane dehalogenase	200-fold longer half-life at 60 °C	Directed evolution	Hydrolysis of lindane	Floor et al. 2014
TIM-barrel proteins	Thermostable	De novo		Huang et al. 2016a, b
Peroxidases	Thermostable	De novo	Reduction of H2O2	Watkins et al. 2017
α-Helical barrel	Introducing new activities	De novo	Hydrolyses pnitrophenyl acetate	Burton et al. 2016
Retro-aldol enzymes	Active site for Multiple reactions	De novo	Explicit H_2O	Jiang et al. 2008
Glyoxalase synthase	70-fold higher activity	De novo	Synthesis of acetyl-CoA	Dinmukhamed et al. 2021

performance for less conserved sequences and high background noise (Fodor and Aldrich 2004). MSA can be curated using T-Coffee, Mafft, or Muscle programs individually. Using such MSA curation, seven residues were identified as coevolved residues in 3-deoxyD-manno-octulosonate 8-phosphatesynthases (KDO8PS) (Astl et al. 2019). Once the customization of the functional sites is established, their 3D models are generated using either (i) homology modelling that is based on comparative modelling or threading by using a homologous protein with experimentally verified 3D structure (Wiltgen 2018) or (ii) ab initio modelling which does not rely on preexisting homologous protein structure (Chakraborty et al. 2018). The specific method and design strategy of natural biocatalysts for optimizing various enzymes are shown in Table 3.

The MSA can be further refined in the "integrated system" by using the noise-based information as evolutionary information obtained from the MSA, such as the gaps and SNPs to improvise the rational enzyme design and performance (Li et al. 2012a, b). The mutations observed through MSA can be used to compensate for the negative effects of one mutation by introducing other mutations in the same coevolving residue pair. The integrated system also inspects for possible improvements such as faster protein folding, catalyzation, and signalling. It also aims to increase the protein stabling by introducing amino acids such as proline and developing disulfide bonds (Lancaster et al. 2018). The rational design also aims to modify the charged residue positions in the binding sites to modify the cofactor or substrate specificity.

Semi rational design Site saturation mutagenesis (SSM) is an efficient approach for directed evolution (Qu et al. 2020). It essentially involves the replacement of target resides with other amino acids. It is commonly performed with degenerate primers with a randomized codon (Siloto and Weselake 2012). A library of all possible mutations in a predetermined position can be generated using this technique. Implementation of SSM on a lipase, from *Bacillus subtilis*, with 181 amino acids generated 181 libraries. Screening of

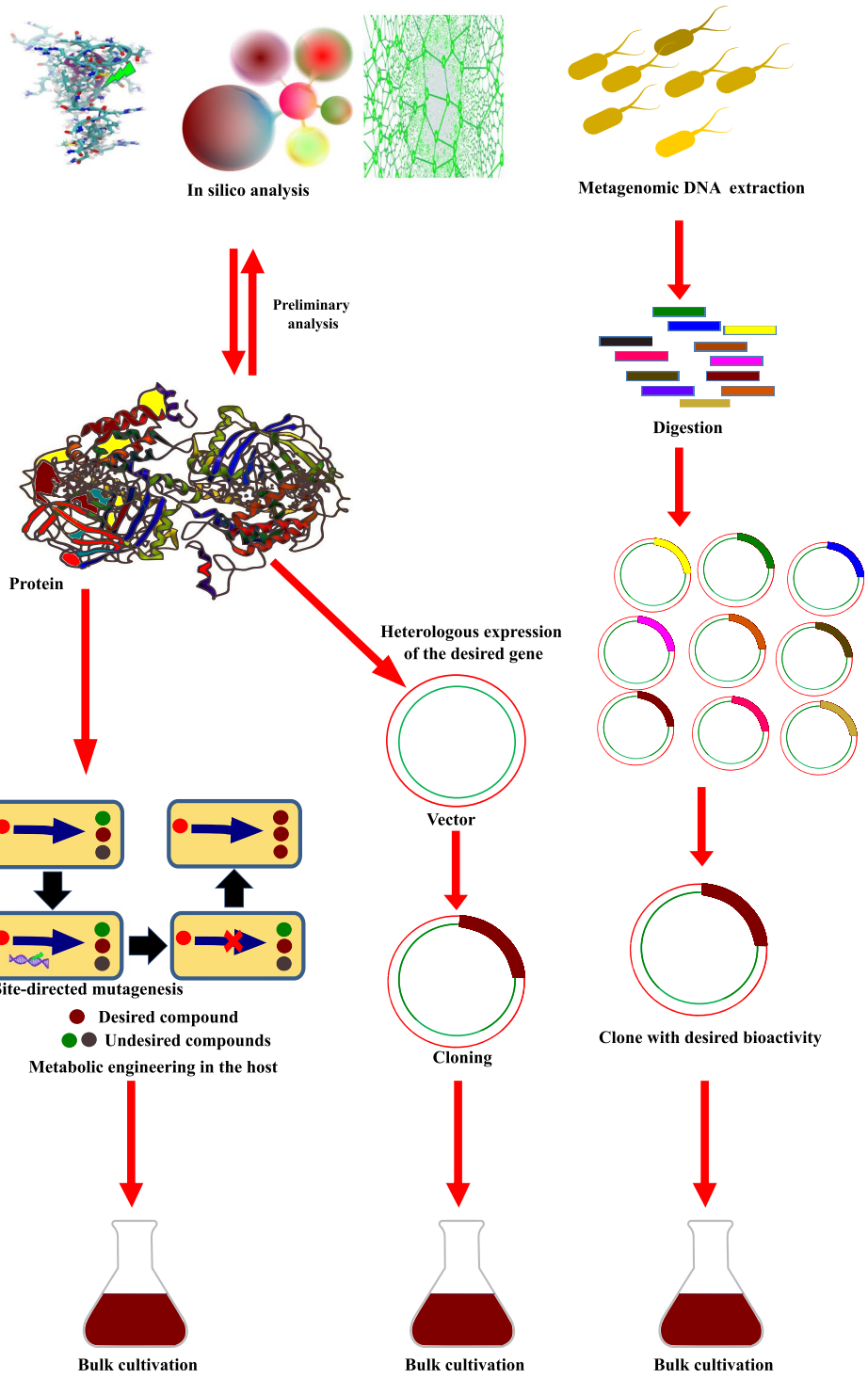

Fig. 3 A standard rational engineering method for hyper-production of desired compounds using conventional in silico and metagenomic approach

the libraries resulted in a novel nitrilases variant (Chica et al. 2005; Shen et al. 2021).

Combinatorial active-site saturation test (CAST) Enhancement of the enzymes is also carried out by using saturation mutagenesis (SM) targeting the active site to increase the substrate acceptation of an enzyme (Georgescu et al. 2003; Reetz et al. 2005). For this purpose, Combinatorial active-site saturation test (CAST) was developed to introduce SM to increase the substrate of an enzyme. In this technique, amino acids with side chains close to the binding sites are selected for random repositions (Qu et al. 2019). CASTing can be in cycles wherein the enhanced enzyme is used as the template for another cycle of CASTing (Osbon and

Kumar 2019). The CASTing can also produce mutations with a synergetic effect when multiple sites undergo SM simultaneously; however, this can exponentially increase the library size (Georgescu et al. 2003).

Iterative saturation mutagenesis (ISM) ISM technique is based on semi-directed evolution involving SM. In this technique, the primary step is identifying sites with single or multiple amino acids. The selected sites are then mutated and processed to screen improved variants (Qu et al. 2021). The variant is subsequently used as a template for saturation mutagenesis at multisite. Thus, it involves variant library generation, screening, and enrichment of the variant for multiple cycles. Although the steps involved in ISM are similar to that of regular directed evolution (Sayous et al. 2020), it differs substantially in selecting the sites and thus is lower required for library screening.

Focused rational iterative site-specific mutagenesis (FRISM) The development of CAST and ISM has greatly assisted in the generation of novel variations with enhanced properties. However, both techniques require screening of many variations and libraries, which can be time-consuming and cost-ineffective (Li et al. 2020; Qu et al. 2020). Hence, FRISM was developed to avoid library generation to increase the turnaround time and be cost-effective. In FRISM, the initial step is the section of a site with the aid of the CAST technique (Acevedo et al. 2020). The amino acid in the template is exchanged with a set of few amino acids predicted with the help of tools used in rational designs and other bioinformatics tools (Höhne et al. 2010). Hence, only a few variants are generated that can be screened rapidly. The next step is the selection of the best variant to be used as the template for mutation at another CAST site.

De novo enzyme design Computational tools have a central role in predicting the 3D structure and functions of a given amino acid sequence that is not of natural origin. The de novo design of proteins was first demonstrated in 1998 by preparing four-helix bundle protein (Bolon et al. 2002; Cochran et al. 2005). De novo enzyme designing requires understanding physical principles involved in protein folding (Baker 2019). Advancement in bioinformatics has made it possible to predict the structure from any amino acid sequence with high accuracy at the atomic scale. Hence, de novo design represents a potential approach to design enzymes from scratch, instead of modifying naturally occurring enzymes, to address the challenges faced in the industrial and biomedical industries.

The most common computational tools for de novo design are the Rosetta developed in the David Baker laboratory (Dou et al. 2018; Richter et al. 2011). It is primarily a package of multiple tools such as protein design, docking, structure, and interaction prediction for DNA and proteins. De novo design process in Rosetta has four main stages: (i) formulation of the optimal catalytic mechanism and ideal active site, (ii) identification of active site localization in a scaffold protein from a scaffold library, (iii) optimization of the residues to obtain stable interaction(s) with primary catalytic residues, and (iv) assessment and rank based designing of the sequences (Richter et al. 2011). In addition, de novo design could optimize the electric field by introducing amino acid substitution to achieve free energy stabilization (Vaissier and Head 2019). The lowest-energy state of the designed structure is a crucial step in the designing process. The de novo designed enzyme is processed for characterization only if the structure-prediction calculations align with the developed system (Huang et al. 2016a). De novo design, in addition to industrial biocatalyst (Table 3), has also been proposed as an ideal tool for formulating peptides and small molecules for therapeutic applications (Bellows and Floudas 2010).

The infinitely large number of possible combinations of amino sequences and their 3D conformations is a major challenge for the formulation of algorithms to predict structure–function. However, the development of novel techniques such as omics, sophisticated databases, and machine learning models has helped advance enzyme designing such as de novo design by finding patterns in sequence 3D structures.

High-throughput avenues for enzyme technology

In the field of enzymology, high-throughput screening (HTS) methods are emerging, and resourceful avenues have expanded into a multi-level approach for enzyme designing and selection with the help of vast genomic, transcriptomic, proteomic, and metabolomic resources (Mazurenko et al. 2020; Yi et al. 2021). Directed evolution, rational, or de novo design are the classical approaches for enzyme engineering, which are faced with significant challenges due to the infinite possibilities in combinatorial arrangements, which is further complicated by the lengthy protein sequence (Bonk et al. 2019). The traditional experimental and computational techniques combined with advanced next-generation high-throughput technology can unleash exciting methods for generating novel enzymes (Gupta et al. 2021). Novel enzyme discovery and development can be accomplished by using various computational and contemporary methods, as mentioned in Fig. 4.

Machine learning in enzyme biology Machine learning (ML) is distinct from the traditional methods of rational designing, directed evolution, or de novo enzyme

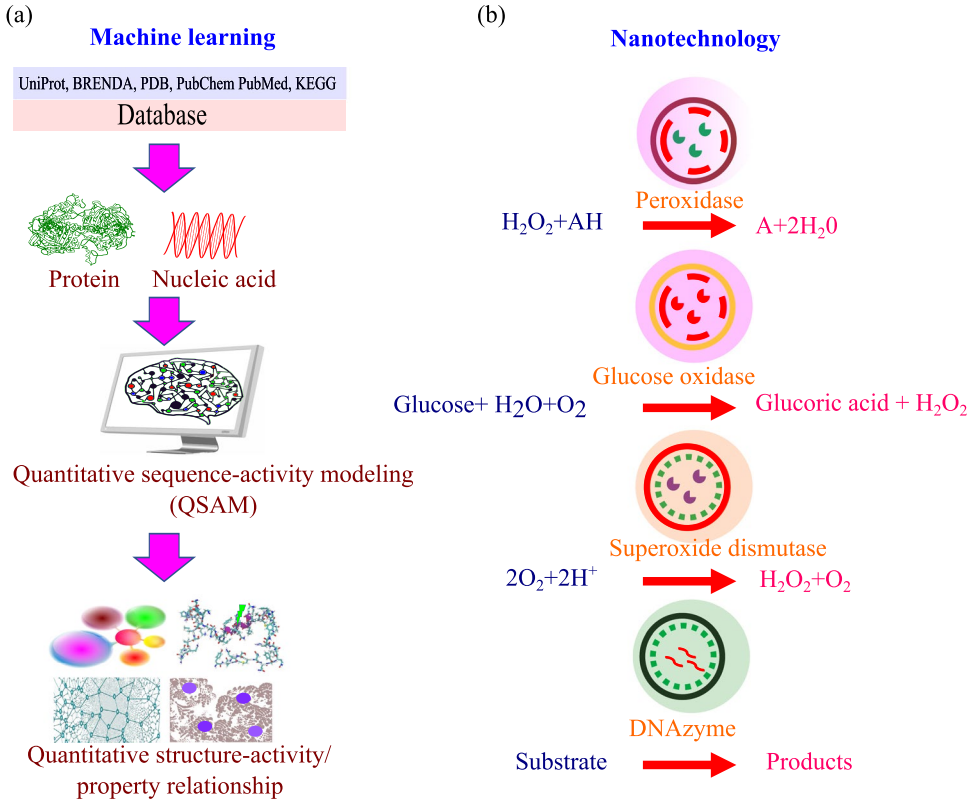

Fig. 4 Schematic illustration of novel enzyme designing through **a** development of machine learning models and **b** nanotechnology for antioxidant, oxidase, and biocatalysis with nano-encapsulation

designing (Mazurenko et al. 2020; Siedhoff et al. 2020). Machine learning models are implemented to analyze the key properties required to develop suitable enzymes, such as the relationship between substrate and enzyme interactions (Ralbovsky and Smith 2021; Siedhoff et al. 2020). Various protein sequence databases, such as UniProt, BRENDA, PDB, PubChem, PubMed, and KEGG, are publicly available. Machine learning models can infer enzymatic properties such as quantitative structure–activity/property relationship (QSAR/QSPR) and quantitative sequence-activity model (QSAM) from such databases. Improving the activity of enzymes is one of the major objectives in protein engineering. As a proof of concept, Liao et al. (2007) produced several variants of proteinase K using machine learning models. These variants were reported to exhibit ~ 20-fold higher activity than the wild type.

Machine learning models can also predict essential genes that can be used as a drug target. Plaimas et al. (2010) have implemented such an approach by construction machine learning models with metabolic networks and genome-wide knockout data. The authors were able to identify 35 enzymes as potential drug targets in *Salmonella typhimurium*. Another critical challenge in enzyme design is the accuracy in identifying active metal-binding sites. Such identification is critical since more than 40% of the metalloenzymes are capable of performing all classes of enzymatic reactions. To apprehend this challenge, Feehan et al. (2021) developed a decision-based machine-learning model that was able to identify protein-bound metal as enzymatic with 92.2% precision. This model can be used to study enzymatic mechanisms and design novel enzymes. ML models can also derive critical information from protein sequences such as enantioselectivity, thermostability, catalytic activity, specificity, and physicochemical features such as conservation information, secondary structure, and amino acid composition (Bonk et al. 2019; Gupta et al. 2021).

In enzyme engineering biology (EEB), novel technologies such as ML can help to discover or design novel enzymes from the huge data available in various data libraries generated through genomic, transcriptomic, proteomic and metabolomics (GTPM) (Tan et al. 2019; Yi et al. 2021). However, one major obstacle in machine learning is the lack of machine-friendly databases. In addition, some of the databases are mis-annotated, populated with disproved results, or no longer maintained. Hence, choosing the right datasets for machine learning is critical to avoid feeding inaccurate data for model development. Although several excellent databases are widely available, it is important to follow the popular FAIR principles of data entry in databases, i.e., findable, accessible, interoperable, and reusable (Mazurenko et al. 2020). This would ensure better data usage with minimal efforts for manual data tidying and ease in data formatting suitable for different projects.

Applications of nanotechnology as biocatalysts Nanotechnology has emerged with strong potential applications in enzyme engineering (Singh et al. 2019b). Nanomaterials are highly sensitive and are already developing to detect the Ebola virus through Nanozymestrip that offers ~ 100-fold higher sensitivity than standard strips (Duan et al. 2015). MnO_2 nanosheets have also been used for gene silencing by releasing Mn^{2+} ions in the presence of intracellular glutathione (GSH) (Fan et al. 2015). Nanotechnology has found numerous applications in medicine and health systems, biosensing, clinic diagnostic, and high-value enzymes (Zhu et al., 2021; Chen et al., 2021).

Nanoparticles have been postulated to be inert yet possess biological activity. For instance, they have antioxidant activity similar to that of peroxidases, superoxide dismutase, and such antioxidants (Gao and Yan 2016). Nanoparticles with enzymes characteristics are known as nanozymes. They have efficient biological activities such as hydrolyzing toxic organophosphates (Singh 2019a). Nanozymes have important applications in genetic testing, diagnosis, drug discovery, environmental surveillance, and food safety inspection (Wang et al. 2019; Elegbede and Lateef 2021).

Enzymes can be immobilization-using nanotechnology while still preserving the catalytic activity by preventing unfolding or aggregation (Singh et al. 2019b). This can be further extended to store enzymes in nano-sized solid, liquid, and gel media (Soriano 2018). Nanomaterials including nanostrips, nanoparticles, nanofibers, nanoballs, nanodots, nanosheets, nanotubes, nanocapsules, and single enzyme nanoparticles (SENs) are also available for efficient enzyme storage without losing their catalytic activity and physicochemical properties. Furthermore, such nanomaterials can be reused; hence, they are eco-friendly and cost-effective (Sukumaran et al. 2015; Soriano 2018). Enzyme immobilization using nanopolymeric matrix, nanocarriers, and nanoparticles can enhance efficiency and specificity (Das et al. 2020). Enzymes in special solid structures would maximize their environmental stress tolerance, specificity, catalytic activity, selectivity, reusability, and recovery.

Nanomaterials can also be used to encapsulate enzymes, a process termed nanoencapsulation, to protect from external degradations and enhance their activity. Nano-encapsulation can transport and release molecular cargos such as DNAzyme into targeted cells for activating specific biocatalytic reactions (Wang et al. 2019). Nanoparticle encapsulation of superoxide dismutase (SOD-NPs) was shown to be highly effective in treating ischemia–reperfusion injury. SOD-NP-treated mice showed 75% survival rate without losing important neurological functions, maintained blood–brain barrier integrity, lowered ROS formation, and prevented apoptosis of neurons (Reddy and Labhasetwar 2009).

Nanozymes can also be enhanced by conjugating with amino acids. For instance, Fan et al. (2015) conjugated Fe_3O_4 nanozyme with histidine that strengthened the affinity to the substrate and ~ 20-fold stronger antioxidant activity. Single-atom nanozymes has been used to mimic the spatial structure of natural cytochrome P450 active centers that showed high oxidase-like activity (Huang et al. 2019). So far, 540 nanozymes of potential applications in various sectors have been developed from 350 research groups in different countries (Meng et al. 2019).

OMIC approaches to find novel enzymes OMIC technology comprises genomics, transcriptomics, proteomics, and metabolomics that analyze the overall genome, transcripts, proteins, and metabolites without any specific target. Hence, OMIC technology provides an unbiased view of the total contents of a sample (Horgan and Kenny 2011). OMIC techniques rely on technological advancements, and it is now widely implemented for high-throughput, cost-effective, and time-efficient generation of genome, expression, and enzymatic profiles (Yang et al. 2020). Although OMIC technology is still in its infancy, it has already contributed tremendously to enzyme discovery, interrogating the entire pool of transcripts, proteins, metabolites, and genome (Table 2) (Ebrahim et al. 2016; Tatta et al. 2021). This also leads to the generation of an incredible amount of nucleic and protein sequences in databases and repositories, which are the foundation for novel enzyme discovery (Zaparucha et al. 2018). Several terabytes of genomic and proteomic data are actively used for novel enzyme design and discovery. For instance, using high-throughput proteomics, the primary metabolism in *Streptomyces coelicolor* for carbon control were found to be regulated by glucose kinase-dependent and independent pathways (Gubbens et al. 2012). Genomic-based novel enzyme discoveries involve the generation of clones libraries from the fragmented genomic sequences followed by an expression in suitable vectors such as a plasmid, cosmid, and lambda to express the desired protein. Implementation of such multi-OMIC techniques has helped in the discovery of enzymes with immense implications in biotechnology, bioremediation, food, and medical industries such as lipases (EC 3.1.1.3) (Vorapreeda et al. 2016), cellulase (EC 3.2.1.4) (Hassan et al. 2018), laccase (EC 1.10. 3.2) (Sirim et al. 2011), and dihydrodipicolinate synthase (EC 4.3.3.7).

OMICs has also advanced the implication of microbes and their metabolites in bioremediation. Through OMICs technology, novel biocatalysts and biomarkers can be identified to monitor toxic contaminants in sewage, garbage, landfills, and industrially polluted water logs (Pandey et al. 2019). It has also helped identify several enzymes capable of degrading toxic compounds in the environment (Madhavan et al. 2017). For example, *Brevibacterium epidermidis* EZ-K02 was found to degrade benzoate, catechol, arsenic, cobalt, and cadmium. Similarly, nitrate and uranium waste were metabolized by *Anaeromyxobacter* sp. (Tatta et al. 2021).

The advanced OMICs tools and related analytical techniques have tremendous scope in nutritional science. OMICs has also contributed to the advancements of personalized or precision nutrition by emphasizing multiple factors, including food quantity, food analytics, nutrition-based diseases, and public health programs. Instead of population-based dietary recommendation, DNA-based dietary recommendation results in greater nutrient absorption from the diet (Bush et al. 2020). However, the high cost of OMIC tools and instruments is prohibitive for the public to access the personalized nutrient technology. Hence, further challenges would be for superior tools and models and at an affordable price tag.

Ultra-high-throughput technologies have an immense implication on healthcare. OMICs have an indispensable role in mapping thousands of genetic variants, which can be automatically processed with computational pipelines to associate such variations with underlying diseases and disorders (Hasin et al. 2017). It has also helped in the development of next-generation disease diagnostics tools such as for tuberculosis (Haas et al. 2016) and non-alcoholic fatty liver disease (NAFLD) (Perakakis et al. 2020).

Conclusion

Microbial enzymes are an indispensable part of the current industrial and healthcare processes. They have been used since the dawn of ages. However, the ever-increasing requirement for high yield and specificity enzymes has surpassed the supply. Harnessing microbial enzymes requires a novel approach different from the traditional methods. The development of novel methods for screening and modifying enzymes is of acute need. Integrating classical and advanced technology is a new avenue for efficient research in the bioprospecting of enzymes. Hence, it would be appropriate to encourage academia and industry research to pave novel techniques to tap the potential of un-culturable microbes. In addition, the development of algorithms and artificial intelligence for bioprospecting enzymes from the publicly available genome and metagenome data is the need of the hour.

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Declarations

Ethics approval and consent to participate This article does not contain any studies with animals performed by any of the authors.

Conflict of interest The authors declare no competing interests.

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