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Applications of Microbial Enzymes in Food Industry

Introduction

Microorganisms have been used in food fermentation since ancient times and fermentation processes are still applied in the preparation of many of the food items. Microbial enzymes play a major role in food industries because they are more stable than plant and animal enzymes. They can be produced through fermentation techniques in a cost-effective manner with less time and space requirement, and because of their high consistency, process modification and optimization can be done very easily. Many of these enzymes find numerous applications in various industrial sectors, e.g. amylolytic enzymes find applications in food, detergent, paper and textile industries. They are used for the production of glucose syrups, crystalline glucose, high fructose corn syrups, maltose syrups, etc. In detergent industry, they are used as additives to remove starch-based stains. In paper industry, they are used for the reduction of starch viscosity for appropriate coating of paper. In textile industry, amylases are used for warp sizing of textile fibres. Similarly, enzymes like proteases, lipases or xylanases have wide applications in food sectors. The following sections give detailed and updated information about various food enzymes of microbial origin.

α-Amylases

α-Amylases (EC 3.2.1.1) are starch-degrading enzymes capable of hydrolyzing α-1,4 glycosidic bonds of polysaccharides, which results in the production of short-chain dextrins. These enzymes are widely distributed in all living organisms. Majority of α-amylases are metalloenzymes and require calcium ions for their activity, stability as well as integrity.

Wide applications of α-amylases in food industry include baking, brewing, starch liquefaction as well as a digestive aid. They are widely used in baking industry as flavour enhancement and antistaling agent to improve bread quality. During baking, α-amylases are added to the dough for conversion of starch to smaller dextrins, which are subsequently fermented by yeast. It improves the taste, crust colour and toasting qualities of bread.

α-Amylases are also used in the manufacture of high-molecular-mass branched dextrins. They are used as a glazing agent for the production of rice cakes and powdery foods. In starch industry, they also find application for starch liquefaction, which converts starch into glucose and fructose syrups. Enzymatic conversion of starch involves three steps: gelatinization, liquefaction and saccharification. Gelatinization involves formation of a viscous suspension by dissolution of starch granules. This is followed by a liquefaction process, which reduces viscosity and involves partial hydrolysis. Glucose and maltose are further produced by saccharification. This requires highly thermostable enzymes and most of the starch saccharification is carried out with α-amylases from Bacillus amyloliquefaciencs, Bacillus stearothermophilus or Bacillus

licheniformis. For the production of ethanol, starch is converted to fermentable sugars by the action of α-amylases and further fermentation of the sugars to alcohol is carried out by Saccharomyces cerevisiae. Other applications of α-amylases include clarification of fruit juices, which is carried out in the presence of cellulases and pectinases to improve yield as well as to make the process cost-effective.

Glucoamylases

Glucoamylases (EC 3.2.1.3) are exo-acting enzymes which catalyze the hydrolysis of polysaccharide starch from the non-reducing end, releasing β-glucose. They are also called saccharifying enzymes and are widely distributed in all living organisms. These enzymes are produced mainly by Aspergillus niger and Aspergillus awamori, but the one produced by Rhizopus oryzae is widely used for industrial applications. Majority of glucoamylases are stable at low temperature. At higher temperatures, they lose activity due to conformational change. Glucoamylases find wide range of applications in food industry, such as for the production of high-glucose syrups and high-fructose syrups. They also find application in baking industry to improve flour quality, reduce dough staling, as well as to improve bread crust colour and the quality of high fibre baked products. Glucoamylases convert the starch present in the flour to maltose and fermentable sugars. Fermentation by yeast leads to dough rise. These enzymes are also used for the production of glucose, which upon fermentation with Saccharomyces cerevisiae yields ethanol. Glucoamylases play an important role in the production of sake and soya sauce, as well as in the production of light beer. They metabolize dextrins and convert them to fermentable sugars with reduced calorific value and alcohol content in the beer.

Proteases

Proteases are enzymes which catalyze the hydrolysis of peptide bonds present in proteins and polypeptides. They are widely used in detergent and pharmaceutical, followed by food industries. They represent 60% of industrial enzymes on the market . The global demand for protease enzyme market has been growing at a compound annual growth rate (CAGR) of 5.3% during the period 2014-2019. Their demand is expected to increase much further as they find applications in leather processing as well as bioremediation processes. Proteases can be classified based on their origin, catalytic activity and nature of the reactive group in the catalytic site. The major sources of protease enzymes are animals, plant and microorganisms (both bacterial and fungal). Proteases are divided into two groups: exopeptidases and endopeptidases, based on the site of action on polypeptide chains. The exopeptidases act on the ends of polypeptide chains and endopeptidases act randomly in the inner regions of polypeptide chains. The endopeptidases are further classified into six groups, based on the catalytic residue presents in the active site: serine, aspartic, cysteine, metallo, glutamic acid and threonine protease. Plant proteases such as bromelain, ficin and papain is widely used in food industry for various applications such as brewing, tenderization of meat, coagulation of milk and as a digestive aid. In addition, proteases are also used to improve the flavour, nutritional value, solubility and digestibility of food proteins as well as to modify their functional properties including coagulation and emulsification. Proteases are widely used in baking industry for the production of bread, baked foods, crackers and waffles. These enzymes are used to reduce the mixing time, decrease dough consistency and uniformity, regulate the gluten strength in bread and to improve the texture and flavour. The acid protease from *Aspergillus usamii* has been successfully employed for the improvement of

functional properties of wheat gluten. The addition of protease could release sufficient peptides and amino acid levels in the wort to get a proper fermentation. Acidic fungal proteases are used in improving fermentation of beer as they are efficient even at low pH by balancing the amino acid profile of beer. Another major application of proteases is associated with dairy industry. Naturally occurring proteases contribute significantly to the flavour characteristics of cheese. They are used for the acceleration of cheese ripening, to modify the functional properties and reduce the allergenic properties of milk products. In cheese making, proteases are also used to hydrolyze the specific peptide bond to generate paracasein and macropeptides (49).

Lactase (β-Galactosidase)

Hydrolysis of lactose is an important biotechnological process in food industry (50). The enzyme β-galactosidase catalyzes the hydrolysis of lactose. It belongs to the family of hydrolases. β-Galactosidase can be obtained from numerous biological systems including plants, animals and microorganisms (51). The production of β-galactosidase from microorganisms such as bacteria, fungi and yeast is a preferred choice due to higher yield and thus relatively low cost of the enzyme (13, 52). The choice of source depends on the final application of the enzyme β-galactosidase, e.g. β-galactosidase from yeasts with pH optima of 6.5-7.0 is generally used for the hydrolysis of lactose in milk of whey. In the case of acidic whey hydrolysis, fungal β-galactosidase with pH optima of 3.0-5.0 is suitable. Thus the selection of β-galactosidase depends on the final application of the enzyme or industry (53, 54). β-Galactosidase produced from yeast Kluyveromyces lactis requires ions such as Mn or Na , whereas Kluyveromyces fragilis requires Mn , Mg or K (55).

In industrial applications, two major classes of β-galactosidase are of prime importance. They are cold-active and thermostable β-galactosidase (56, 57). On commercial scale, β-galactosidase is produced using microorganisms with GRAS status for their application in milk and dairy products. Lactase is used with milk and milk-based products to reduce lactose intolerance in people. The scoopability and creaminess of ice creams improved significantly after the hydrolysis of lactose with lactase (50). Additional advantage of hydrolyzing lactose into monomers is the reduction requirement of sweeteners as they could improve the sweetness of the products (13, 58). Another major application of lactase is the lactose hydrolysis in whey. Whey is a By-product of cheese production and its main components are lactose, proteins and minerals. This causes critical environmental issues associated with dairy industry as lactose is associated with high biological oxygen demand (BOD) and chemical oxygen demand (COD) (59, 60). Another application of lactase is the formation of galactooligosaccharides (GOS) from lactose hydrolysis due to transglycosylation activity of β-galactosidase. The GOS could be used as prebiotic food ingredients (14).

Lipases

Lipases are enzymes which catalyze the hydrolysis of long-chain triglycerides. They are naturally present in the stomach and pancreas of humans and other animal species in order to digest fats and lipids (61). Microbial lipases are produced by bacteria, fungi and yeast. Microbial enzymes contribute to approx. 90% of global lipase market (62). This enzyme finds application in various industries including food, biofuel, detergents and animal feed. It is also used in leather, textile and paper processing applications (63). In the food and beverage industry, lipases find major application in dairy, baking, fruit juice, beer and wine industries. Although it finds many applications in various

industries, the market share of lipase is less than 10% of global industrial enzyme market (62).
Commercial lipases are mainly used for flavour development in dairy products and processing of other foods containing fat (16). They can improve the characteristic flavour of cheese by acting on the milk fats to produce free fatty acids after hydrolysis (15). Different types of cheese can be made by using lipases from various sources, e.g. Romano cheese using kid/lamb pre-gastric lipase, Camembert cheese using lipase from Penicillium camemberti and cheddar cheese using Aspergillus niger or A. oryzae (16). Lipase catalysis could improve the texture and softness of cheese. Lipases are also used as flavour development agents in butter and margarine, also to prolong the shelf life of various baking products (16). In alcoholic beverages such as wine, the aroma can be modified using lipase. They are used to improve the quality of cocoa butter, which has a melting point of 37 °C due to the presence of palmitic and stearic acids and can easily melt at 37 °C (64, 65). A patent has been filed by Unilever using immobilized Rhizopus miehei lipase, which can replace palmitic with stearic acid to give desired stearic-oleicstearic triglyceride (64-66). Functionalized phenols were esterified for the synthesis of lipophilic antioxidants for the application in sunflower oil using immobilized lipase from Candida antarctica (CALB), Candida cylindracea Ay30, Helvina lanuginosa, Pseudomonas sp. and Geotrichum candidum. Lipases also find application as a biosensor in food industry. Immobilized lipase was successfully used for the determination of organophosphorous pesticides with a surface acoustic wave impedance sensor by lipase hydrolysis (67). It may also be used in the determination of triglycerides and blood cholesterol if the lipase is immobilized onto pH/oxygen electrodes in combination with glucose oxidase (68). Microbial lipases such as lipase from Candida rugosa have many applications which cannot be met by chemical synthesis. This lipase finds application in the production of ice cream, single-cell protein, carbohydrate esters and amino acid derivatives (16). In addition to this, lipase could also be used in the processing of different waste streams that are released from food industries (69).

Phospholipases

Phospholipases selectively break down phospholipids into fatty acids and other lipophilic substances. They can be divided into four major classes (A, B, C and D) based on their mechanism of action (70). Phospholipase A1 (PLA1), phospholipase A2 (PLA2), and phospholipase B act on the carboxylic ester bonds of phospholipids, thus displacing and replacing the acyl group chain through various chemical reactions like hydrolysis, esterification and transesterification. Phospholipases C (PLC) and D (PLD), which modify polar head group, are also known as phosphodiesterases, and they recognize the phosphodiester linkage (70). Phospholipases are widely used in food industry, most importantly in the production of oils, dairy industry and in the manufacture of several bakery items (71). They also find applications in the degumming of various vegetable oils, cheese manufacture and bread manufacture (71). Phospholipase from Fusarium oxysporum is a commercially available phospholipase which has both phospholipase and lipase activities and it is marketed by Novozymes A/S (Denmark) for baking application under the name LipopanF® (71). The PLA2 commercialized by DSM Food Specialties (The Netherlands) with the trade name Maxapal® A2 has been reported by Zhao et al. (72), who described that the egg yolk treatment with PLA could increase the stability of dough and interaction of starch and gluten. Another commercial phospholipase in baking industry is LysoMax® product (DSM Food Specialties), which is made up of a bacterial strain, specifically acting on lecithin (73). Phospholipases are also used in the processing of various dairy products to enhance the stability of fat or

maximise the yield of cheese, milk, butter and ice cream (71). The important applications of lipases include enhancing the cheese flavour, lipolyzed milk fat production for use in butter as flavour, etc. (17).

Esterases

In aqueous solution, esterases are able to facilitate the splitting of esters into acid and alcohol. In addition to this, esterases hydrolyze short-chain rather than long-chain acylglycerols, thus being different from lipases. Esterases play a prominent role in the food industry and alcoholic beverage industries, where they have been mostly used for the modification of oil and fat in various fruit juices and to produce fragrances and flavours (18). Feruloyl esterases, an important group of enzymes from esterase family, break the ester bond between ferulic acid and different polysaccharides in plant cell wall. Since feruloyl esterases hydrolyse lignocellulosic biomass, they are inevitable for waste management (19).

Cheng et al. (74) screened for feruloyl esterase activity in a metagenomic library obtained from the microbial population of a cow rumen and identified a proteaseresistant feruloyl esterase, which can release ferulic acid from wheat straw. This particular esterase has great commercial application because of its high pH and thermal stability and protease resistance.

In cheese manufacture, the fruity flavours are the result of different methyl or ethyl esters of short-chain fatty acids. Bacterial production of ethyl esters and thioesters has been reported. Alvarez-Macarie and Baratti (20) reported the production of a novel thermostable esterase from the highly thermotolerant Bacillus licheniformis heterologously expressed in E. coli for the production of short-chain flavour esters. Feruloyl esterase is a key enzyme in the biosynthesis of ferulic acid, which is the precursor for vanillin, an aroma compound used in foods and beverages (75). Several researchers have reported the microbial production of ferulyl esterase (76, 77).

Lipoxygenases

Lipoxygenases (LOX) are involved in the dioxygenation of polyunsaturated fatty acids in lipids containing a cis-1,4-pentadiene. They contain single polypeptide chain which is further assembled into an N-terminal domain and the catalytic β-barrel domain. LOX enzymes are non-haem iron-containing enzymes. The LOX-catalyzed reaction produces different precursors for the production of different volatile and aroma-producing chemical substances in plants. LOXs are used in aroma generation in food industry and also in bread making (78). Soya bean lipoxygenases (LOX) is the most studied lipoxygenase enzyme. Bacterial LOXs possess different specificity towards fatty acids. LOX from Nostoc sp. oxygenates at specific site in linoleic acid, but the LOX from Anabaena sp. exhibits variable specificity (79-82).

The main applications of LOXs in dough are based on their ability to bleach the flour pigment carotenoid, by co-oxidation of the pigment with fatty acids (83, 84). Lipoxygenases are also employed to improve tolerance to mixing and different handling properties of dough (78). This effect is due to the oxidation of thiol group in gluten, which may lead to redistribution of different disulphide bonds, tyrosine crosslinking and subsequent strengthening of the gluten. This also leads to the improvement in dough rheology. Recently Patel et al. (85) purified lipoxygenase from Lasiodiplodia theobromae by different chromatography techniques and fully characterized the enzyme. L. theobromae was reported to contain two types of lipoxygenases with molecular mass of 93 and 45 kDa (86).

Cellulases

Cellulases are enzymes that act on polymeric cellulose and hydrolyze β-1,4 linkages to liberate glucose units. The three major classes of cellulases are endo-(1,4)-β-D-glucanase (EC 3.2.1.4), exo-(1,4)-β-D-glucanase (EC 3.2.1.91) and β-glucosidases (EC 3.3.1.21) (87). The catalytic modules of cellulases belong to glycosyde hydrolase (GH) family and have been classified in different groups based on differences in amino acid sequences and three-dimensional structural features. GH family enzymes mainly use acid–base catalysis mechanism for cleaving glycoside bonds in cellulose. The catalysis is achieved by two major residues (a proton donor and a nucleophile) of the enzyme in the active site region (88). The hydrolysis occurs via retention or inversion mechanism depending on the spatial position of these catalytic residues in the enzyme. Endoglucanases cleave β-1,4-bonds in amorphous region of cellulose and expose the non-reducing and reducing ends of cellulosic polymer. Endoglucanases from various sources belong to different glycoside hydrolase families, among which the major are 5–9, 12, 44, 45, 48, 51 and 74. Most of the fungal endoglucanases contain a catalytic module with carbohydrate-binding module (CBM), but catalytic module without CBM was also reported from fungal species (89). Multiple catalytic modules and CBMs are present in bacterial endoglucanases. A cleft/grove-shaped active site is present in the catalytic module of most of the endoglucanases (90). Exoglucanases or cellobiohydrolases (CBHs) act on available reducing or non-reducing ends of cellulose polymer and liberate cellobiose. Fungal and bacterial CBHs show diversity in catalytic module and belong to glycoside hydrolase families 5, 6, 7, 9, 48 and 74 (91). A tunnel-shaped catalytic module is observed in most of the CBHs. β-Glucosidases catalyze the final step in cellulose breakdown by cleaving the non-reducing terminal β-D-glucosyl residues and removing β-D-glucose (92). The catalytic modules belonging to glycoside hydrolase families 1, 3 and 9 are reported from various β-glucosidases. The cellulolytic machinery of microbes is mainly regulated through feedback inhibition of b-glucosidases by their reaction product glucose. The major difference from CBHs is the absence of CBM in their structure. A pocket-shaped active site region of β-glucosidases helps them to attach the glucose molecule to non-reducing end and release glucose unit from cellodextrins or cellobioses (90). A large diversity of microorganisms is reported to produce cellulases during their growth on cellulosic materials. The industrial making of cellulases is mainly from microbial sources, bacteria and fungi, and these microorganisms can be diverse in their habitat. The aerobic bacteria show similar mechanism of cellulose degradation to that of aerobic fungi. In anaerobic bacteria, cellulosomes are located on the cell surface and operate via a different system. Cellulases from fungi (Aspergillus and Trichoderma) and bacteria (Bacillus and Paenibacillus) are potentially used in the production of food. They are also widely used for various industries such as textile, paper, detergent and food industry (21). In juice industry, cellulases are applied in combination with other macerating enzymes for increasing process performance and yield, improving the extraction methods, clarification and stabilization of juices (22). They can also reduce the viscosity of nectar and puree from fruits such as apricot, mango, plum, papaya, pear and peach, and are used for the extraction of flavonoids from flowers and seeds. The preferences of cellulase-mediated extraction over conventional methods are due to higher yield, less heat damage and short processing time. Cellulases are utilized for the extraction of phenolic compounds from grape pomace (93). β-Glucosidases in combination with pectinase alter the structure, flavour and aroma of fruits and vegetables (94). They are also reported to reduce bitterness of citrus fruit and improve aroma and taste (95). Cellulases are used with other enzymes for efficient olive oil extraction (96). In wine production, cellulases are used in

combination with other enzymes to increase yield and quality (97). The main advantages of using these enzymes are improved maceration, better colour development, must clarification and finally wine stability and quality (98). Studies of Oksanen et al. (99) showed that cellulases can significantly reduce wort viscosity. The aroma of wines can be improved by β-glucosidases through modifications of glycosylated precursors.

Xylanases

Xylanases are produced by microorganisms to cleave xylans, a major constituent of hemicellulose. Three major enzymes, endoxylanases, exoxylanases and β-xylosidases, act synergistically and are required for the breakdown of xylan backbone in hemicellulose. Endoxylanases (EC 3.2.1.8) cleave the β-1,4 bonds of xylan backbone. Exoxylanases (EC 3.2.1.37) hydrolyse β-1,4 bonds of xylan from the non-reducing ends and release xylooligosaccharides. β-Xylosidases cleave the xylobiose and xylooligosaccharides to release xylose (100). The major functions of xylanases are performed by a catalytic module and few classes possess an additional CBM for binding to substrates. The two major catalytic modules of hemicellulases are glycoside hydrolases (GHs) and carbohydrate esterases (CEs). Endoxylanase hydrolyses the xylan backbone and has catalytic cores belonging to GH families 8, 10, 11, 30 and 43 with the most common ones being GH 10 and 11 (101). These differ in their substrate specificities and the GH10 is more active on substituted xylan. Similar to cellulases, they may also contain CBMs (102). Exoxylanases randomly cleave the xylan backbone from inside, releasing long chain xylooligomers on which the β-xylosidases act. The catalytic module of these enzymes belongs to the GH families 3, 30, 39, 43, 52 and 54. These two enzymes are often collectively called xylanases. β-Xylosidase or xylan-1,4-β-xylosidase act on the xylooligosaccharides and xylobiose to release xyloses (103).

Xylanases are produced by microbes like actinomycetes, bacteria and fungi. The major actinomycete and bacterial species producing xylanase are Streptomyces sp., Bacillus sp. and Pseudomonas sp. (104-106). Those produced by bacteria and actinomycetes are effective in a broader range of pH (5.0–9.0), with the optimum temperature for xylanase activity between 35 and 60 °C. Fungi are major sources of xylanase due to their high content and extracellular release of the enzyme (107). The major fungal species producing xylanase are Aspergillus sp., Fusarium sp. and Penicillium sp. Fungal xylanases have higher activity than bacteria or yeast (108).

Carbohydrate-hydrolyzing enzymes are usually used in bread making industry. Rheological properties of dough are improved through enzymatic hydrolysis of nonstarch polysaccharides (109). Xylanases are widely used in bread making industry with other enzymes. The potential effectiveness of xylanolytic enzymes increases its use in bread making. They can increase the specific bread volume and this improves the quality of bread. The hemicellulose in wheat flour is broken down by xylanase, which increases the binding of water in the dough. The dough becomes softer and crumb formation is delayed, allowing the dough to grow (108). Xylanase is used to improve texture, tastiness and palatability in biscuits. They also play an important role in juice production by improving extraction, clarification and stabilization (23). In combination with other enzymes, xylanases lead to better yield of juice and increased recovery of aromas, essential oils, vitamins, mineral salts, pigments, etc. (110). In beer making industries, xylanases are used for hydrolysing the cellular wall of barley. Hydrolysis leads to release of arabinoxylans and lower oligosaccharides, which reduces the muddy appearance and viscosity of the beer (24).

Pectinases

Pectinases are enzymes which catalyze the hydrolysis of glycosidic bonds in pectic polymers. Pectic substances found in tomato, pineapple, orange, apple, lemon pulp, orange peel and other citrus fruits act as natural substrate for this enzyme. Functionally pectinases can be categorized as polygalacturonases (which hydrolyse glycosidic α-(1-4) bonds), pectin esterases (which remove acetyl and methoxyl groups from pectin), pectin lyase and pectate lyase (111). Pectinases can be produced from natural as well as recombinant microbes with attempts made to increase their thermostability and yield (112). Pectinases can also act either on smooth or hairy regions of pectin (113). Based on pH, there are acidic and alkaline pectinases also grouped in endopectinases when enzyme cleaves randomly, and exopectinases when the terminal ends are targeted. Pectinases find a multitude of industrial applications such as in paper bleaching, food industry, remediation, etc. (25). Juices with added pectinase have a clearer appearance and filterability than enzyme-depleted counterparts (111). Apart from reducing the turbidity and haze generation of naturally derived fruit juices such as apple and banana, pectinases also improve the colour and flavour of drinks (113, 114). The addition of gelatin and pectin greatly increases the viscosity and turbidity of juices, and removal of the haze is the most costly part of juice production. The use of biogenic enzymes such as pectinases in juices would act almost nine times better than mechanical maceration to get good results.

Glucose oxidase

Glucose oxidase (EC 1.1.3.4) belongs to a large group of enzyme family called oxidoreductases. Glucose oxidase is a flavoprotein discovered in 1928 by Müller (115). He stated that in the presence of dissolved oxygen the enzyme can convert glucose to gluconic acid. In the reaction, β-D-glucose is oxidised to gluconolactone and molecular oxygen is reduced to hydrogen peroxide. The glucanolactone is then spontaneously hydrolysed to gluconic acid (116). The enzyme is homodimeric and contains two similar polypeptide chain subunits (80 kDa). The subunits are covalently linked by disulphide bonds and one flavin adenine dinucleotide (FAD) molecule non-covalently bound to active site region of each subunit. The glucose oxidase production has been reported from various microorganisms and it was first discovered in Aspergillus niger and Penicillium glaucum. Aspergillus niger species is widely used for production of glucose oxidase and its strains can produce higher amount of glucose oxidase (117). Penicillium adametzii is a widely used fungus for the production of extracellular glucose oxidase (118). The different bacterial species are also reported to produce glucose oxidase. Although many species of bacteria and fungi are reported to produce this enzyme, fungi are considered for the industrial production of glucose oxidase (119).

Glucose oxidase has its wide use in various industries like pharmaceutical and food industries, and in biofuel cells (26). Its use is increasing in baking industry because its oxidizing effects make stronger dough (27). In food industry, it enhances the flavour, aroma and stability of food products by removing glucose and oxygen from diabetic drinks and egg white (27). Glucose oxidase improves the colour, texture, flavour and shelf life of food products and prevents rottening (27). During food packaging glucose oxidase is used for increasing storage life by removing oxygen (27).

Laccase

Laccases (EC 1.10.3.2) are a cluster of oxidases which represent the largest subgroup of multicopper enzymes. Commonly known as blue oxidases, they are used for studying their potential to oxidize phenolic compounds and therefore applied in several

industrial sectors (120-122). These enzymes act as a potent biocatalyst for application in chemical synthesis, biobleaching of paper pulp, bioremediation, biosensing, wine stabilization and textile finishing. They have different specificity for substrate and a wide range of oxidizable substrates, which further depends on the type of microbial sources producing the enzyme (121). Laccases catalyze the oxidation of a wide range of compounds such as phenolics, aromatic amines and ascorbate (120, 121). These enzymes combine reducing substrate having four oxidized electrons with four reduced electrons for cleaving dioxygen bond in the presence of four copper atoms present in laccases (120). The mechanism of catalytic activity of laccase is described in the report of Madhavi and Lele (121) and Morozova et al. (122).

Laccases are secreted extracellularly by several fungi as a product of their secondary metabolism during fermentation, but their production is limited to a few fungal species (122). Well known producers of laccases belong to Deuteromycetes, Ascomycetes and Basidiomycetes (123, 124). Funalia trogii is a white rot fungus capable of producing laccase through absorbent fermentation. The maximum laccase production by F. trogii reached 11 900 U/L, which was 4.97 times higher than that of normal fermentation (125). Bacillus licheniformis produces recombinant laccases for industrial applications (126). Recently, heterologous expressions have been used for laccase production. Bacillus vallismortis fmb-103 genes were cloned and heterologously expressed in Escherichia coli BL21 (DE3) cells (127).

Laccase is used for modification of colour appearance of food and beverage industries, or for wine stabilization as an alternative to physical and chemical adsorbents. Removal of polyphenols from wine should be chosen to avoid adverse changes in wine organoleptic characteristics including stability in acidic medium and reversible inhibition due to the presence of sulphite (28). Furthermore, this enzyme is used in cork stopper manufacturing industry (128).

Haze formation is one of the problems in brewing industry. To avoid it, laccases have been applied for polyphenol oxidation as substitute for traditional approach by different researchers (129-131). This enzyme is also used for oxygen removal in the final step of beer production which prolongs the storage life of beer. Commercial laccase called Flavourstar, manufactured by Novozymes, is used for removing the off-flavour formation in brewing industry (132). It is used in baking because it has the capability to cross-link with biopolymers. The application of laccase in baking enhances stability, strength and decreases stickiness which further increases machinability of bread batter. Moreover, it increases volume and enhances softness of the product as reported by Labat et al. (29) and Si (133).

Catalase

Catalase (EC 1.11.1.6) is a tetrameric protein found in aerobic organisms. It helps hydrogen peroxide decomposition. This enzyme can be produced from microbial sources such as Aspergillus niger and Micrococcus luteus and from bovine liver. Microorganisms are usually preferred as sources for enzyme production due to their advantages such as fast growth, easy handling and genetic tuning for obtaining a desired product (134, 135). The anaerobic Bacteroides fragilis exhibited increased catalase levels in media with haem (136). Frankenberg et al. (137) isolated catalase from Enterococcus faecalis, which completely depends on haem source without which it cannot synthesize porphyrin group. A facultative anaerobic catalase-producing Bacillus maroccanus resistant to hydrogen peroxides was isolated from textile effluents (138). A potent catalase-producing bacterium Pyrobaculum calidifontis was isolated from hot springs in Los Banos and Calamba, Laguna, Philippines (139). A thermo-alkaliphilic

catalase-positive strain of Bacillus halodurans LBK 261 was isolated from alkaline hotspring waters of Kenya (140). A halo(alkali)tolerant catalase-producing Oceanobacillus oncorhynchi ssp. incaldaniensis was isolated from an algal mat capable of producing catalase at wide range of pH 6.0–9.5 and salinity of 5–20% (141). A catalase-positive psychrophile Bacillus N2a was isolated from seawater (142). Other catalase-positive bacteria such as Rhizobium radiobacter were isolated from industrial effluent from beverage industry (143), Comamonas testosteroni and C. terrigena from effluent sludge enriched with crude oil along with heavy metals (144) and Serratia SYBC08 from hydrogen peroxide sludge (145). Psychrobacter piscatorii T-3, a psychrotolerant bacterium isolated from bleach-rich runoff, has high catalase activity (146). Fungi and yeast are able to produce catalase. The highest level of catalase activity of 400 mg/g was observed in isolates of Aspergillus niger (144). A catalase-positive entomopathogenic fungus Metarhizium anisopliae strain Ma10 (CNRCB MaPL10) was isolated from Geraeus senilis (147).

In fabric industry, catalase is used for removing excess hydrogen peroxide from fabric. This enzyme is mostly used along with other enzymes in food processing industry. Catalase is often used with glucose oxidases for food preservation. Ough (30) used a glucose oxidase/catalase cocktail for elimination of oxygen from wine before bottling and evaluated the formation of acetaldehydes. Results showed that colour and amount of acetaldehyde were stable if treated properly with enzymes (148). Catalase is applied in milk processing industry to eliminate peroxide from milk (31), to remove glucose from egg white in baking industry and in food wrappers to prevent oxidation and control perishability of food. This enzyme has limited use in cheese production.

Peroxidase

Peroxidases (EC 1.11.1.7) are oxidoreductase proteins that contain iron(III) protoporphyrin IX as the prosthetic group. They catalyse the reduction of peroxides and oxidation of a wide range of inorganic and organic compounds. Their molecular mass ranges from 30 000 to 150 000 Da, and they comprise a group of unique enzymes such as iodide peroxidase, NADH peroxidase and glutathione peroxidase as well as a group of other nonspecific enzymes (149). Peroxidases are present in plants, microorganisms and animals. They are involved in lignification processes in plants (150) and defence mechanisms against damaged or infectious tissues (151).

Among microorganisms, Phanerochaete chrysosporium is the best characterized peroxidase-secreting organism (152). Industrial scale applications of fungal peroxidases are limited by challenges associated with post-translational modification of proteins (153). However, bacterial peroxidases are easier to produce and have better stability and activity suitable for industrial applications. These enzymes are applied with bacterial laccases for dye decolourization (154). Peroxidase activities are reported in bacterial taxa, such as Firmicutes, Proteobacteria, Actinobacteria and Acidobacteria (155, 156). Moreover, actinomycetes, which are soil bacteria, are able to grow like fungi and have similar ecological niche, and can produce peroxidases for lignin degradation (154, 157). The first secreted extracellular lignin peroxidase was produced by Streptomyces viridosporus T7A (158).

Peroxidase catalyzes a wide range of substrates using hydrogen peroxide or other peroxides (159). This enzyme is used in food industry for producing flavour, colour and texture and improving nutritional quality of food. Other applications include as biosensors, in polymer synthesis and in the management of pollutants in the environment (160). It can be used for treating phenolic effluents from industries. Thermal inactivation of peroxidases is used in food industry to measure the efficiency

of blanching treatment, which further enhances the shelf life of food (32). The negative effect of peroxidases is that they cause undesirable browning of fruits and off-flavours of vegetables.

A-Acetolactate Decarboxylase

α-Acetolactate decarboxylase greatly aids in the fast maturation of beer (161). This enzyme can be produced from natural microbes such as Brevibacillus brevis (162) or from recombinant Saccharomyces cerevisiae (163). The enzyme catalytically converts acetolactate to acetoin via a two-step reaction involving direct decarboxylation of substrate to an enol derivative and its further protonation to final product (162). Enzyme-based removal of α-acetolactate and α-aceto-α-hydroxybutyrate assists in overcoming the rate-limiting step of beer maturation. While the maturation of beer without the use of enzymes takes 2 to 12 weeks (33), the use of α-acetolactate decarboxylase results in maturation within 24 hours depending on the source of enzyme. Moreover, the off-taste due to the presence of diacetyl in beer is nullified by the action of this enzyme. Studies indicate that both free and encapsulated form of this enzyme work efficiently in the process, thus aiding the use of immobilized enzymes at reduced costs (163). Novel inorganic nanoflowers or alginate microbeads immobilized with α-acetolactate decarboxylase are promising strategies with better thermal stability, reusability and catalytic efficiency (164).

Asparaginase

Of the various microbially derived enzymes, asparaginases form a major class of pharmaceutical, nutraceutical and industrially significant enzymes widely used by man. Asparaginase, as the name implies, catalyses the breakdown of the asparagine to subsequent acid derivative aspartic acid and NH and can be considered as the asparagine-depleting enzyme. Asparagine is a nonessential amino acid to humans, whereas it is an essential amino acid for cancerous cells. Thus, the depletion of asparagine critically affects the growth of cancerous cells, which forms the basis of this enzyme as anticancer agent.

Various food processing methods such as frying in oil and baking cause the conversion of asparagine to acrylamide, a known carcinogen. Among various methods attempting to overcome the acrylamide formation, the depletion of asparagines by enzymatic treatment has been found effective in reducing the formation of acrylamides from asparagines by 97%.

Debittering Enzymes – Naringinase

Naringinase (EC 3.2.1.40) is mainly responsible for the breakdown of naringin, the principle bitter flavanone glycoside found in citrus fruits. Naringin is broken down to a glycon naringenin and rhamnose as a result of its α-rhamnosidase and β-glucosidase action. Nariginase is produced mostly by fungal isolates, viz. Aspergillus niger, Circinella, Eurotium, Fusarium, Penicillium, Rhizopus and Trichoderma and bacteria such as Bacillus sp., Burkholderia cenocepacia, Bacteriodes distasonis, Thermomicrobium roseum, Pseudomonas paucimobilis, etc. Fungal sources of naringinase are found to be more predominantly used than the bacterial ones due to increased yield.

Naringinase has a major role in food processing as a debittering enzyme supplemented to fruit juices. Both free and immobilized forms of this enzyme are used to get better results. Immobilization of this enzyme has been done in a variety of substrates such as polyvinyl alcohol cryogels, packaging films, cellulose triacetate nanofibre, graphene, etc. Various food additives such as biopolymers and

sweeteners can be synthesized using rhamnosidase or naringinase. Yet, another use of naringinase together with β-glucosidase and arabinosidase is to improve the aroma of wine. The use of naringinase is also noted in tomato pulp preparation, kinnow peel waste treatment and prunin preparation.

Conclusions and Future Perspectives

Enzymes find application in food, detergent, pharmaceutical and paper industries. Nowadays, the enzymatic hydrolysis and enzyme-based processes are preferred to the chemical ones due to the environmentally friendly nature, efficient process control, high yield, low refining costs and process safety. In comparison with plant and animal enzymes, microbial enzymes can be produced very effectively by different fermentation techniques like solid-state and submerged fermentations. It is also easy to produce microbial enzymes on a large scale. The microbial enzymes can be easily modified through various molecular and biochemical approaches. Hyperproduction of microbial enzymes with high specific activity can be achieved by overexpression of their genes. Many of the enzymes of microbial origin are still unexplored and there are many opportunities for finding wider industrial application of microbial enzymes, especially in food sector.

Collected from “Applications of Microbial Enzymes in Food Industry
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