Esterases as emerging biocatalysts—mechanistic insights, genomic and metagenomic, immobilization and biotechnological applications

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Abstract

Esterase enzymes are a family of hydrolases that catalyze the breakdown and formation of ester bonds. Esterases have gained a prominent position in today's World Industrial Enzymes Market. Due to their unique biocatalytic attributes, esterases contribute to environmentally sustainable design approaches, including biomass degradation, food and feed industry, dairy, clothing, agrochemical (herbicides, insecticides), bioremediation, biosensor development, anticancer, antitumor, gene therapy, and diagnostic purposes. Esterases can be isolated by a diverse range of mammalian tissues, animals, and microorganisms. The isolation of extremophilic esterases increases the interest of researchers in the extraction and utilization of these enzymes at the industrial level. Genomic, metagenomic, and immobilization techniques have opened innovative ways to extract esterases and utilize them for a longer time to take advantage of their beneficial activities. The current study discusses the types of esterases, metagenomic studies for exploring new esterases and their biomedical applications in different industrial sectors.

Keywords: Biocatalysis; Esterase; Biocatalytic properties; Genomic and metagenomic; Immobilization; Biomass degradation; Biosensor development

1. Introduction

Esterase influences the rates of reversible reactions, i.e., the organic phase supports ester synthesis that can be broken down by the same enzyme in the aqueous phase (Khodami et al., 2001). On the other hand, Esterases vary from lipases primarily in substrate specificity and interfacial activation (Panda and Gowrishankar, 2005). Lipases have a hydrophobic domain surrounding the active site, prefer long-chain fatty acid triglycerides, whereas esterases have an acyl binding pocket (Shukla, 2012). Esterases are suitable for many commercial processes due to their excellent properties, including high enantioselectivity, broad-spectrum substrate specificity, and high stability in organic suspensions. They have enormous applications in the food industry in flavor-ester production processes; as flavorings represent over one-quarter of the world food additive market, consumers have shown a preference for food products that can be labelled "natural" and biochemically produced flavor ester on the enzymes excel (Ahmed et al., 2010). Different flavor esters have been synthesized using microbial lipolytic enzymes

thus far (Dandavate et al., 2009). However, considering their market importance, esterases seem to be less common than lipases, owing to a lack of availability (Liu et al., 2013).

Various applications of esterases have been published, primarily in dissemination, quantification, processing, directed production, refinement, and molecular biology, as uses for esterases have been identified in several areas due to increasing interest in this enzyme. For essential applications in biological processes, researchers may make this enzyme from *Streptomyces sp., Pseudomonas sp., Bacillus sp., Lactobacillus sp., Thermoanaerobacterium sp., Micrococcus sp., Ophistoma sp., Penicillium sp., Aspergillus sp., Humicola sp* (Panda and Gowrishankar, 2005). The esterases generated by various sources differ. Again, the substrate used for the testing of related esterases from many sources, for instance, cholesterol esterase testing, has been used for either cholesteryl linoleate (Nishimura and Inouye 2000), cholesterol palmitate (Riley et al., 1990), or p-nitropheline acetate (Finer et al., 2004). Esterases come in a variety of forms. In certain cases, high purity is not needed. As a result, each enzyme would need a unique purification technique. As a result, reviewing some aspects of esterase currently is counterproductive. The vast range of applications for esterase and the increasing demand for it can be met by developing appropriate production strategies (Panda and Gowrishankar, 2005). As a result, identifying, isolating, and characterizing novel esterases with distinct properties are crucial for industrial use. Esterases in different mammalian tissues, plants, and microorganisms are widely spread (Fahmy et al., 2008). Since they are more soluble and much cheaper to manufacture on a wide scale, microbial esterases have gotten a lot of attention from the industry (Liu et al., 2013).

2. Structural and mechanistic insights of esterases

Esterase enzymes are part of the lipolytic hydrolase family, and in a range of substrates, they catalyze the cleavage and form ester bonds. Most esterases in alpha/beta hydrolase folded protein (Pfam PF00561 domain) belong to the superfamily (Punta et al., 2012) of carboxylesterase gene families (Hotelier et al., 2010). Various enzymes can hydrolyze a broad range of substrates in the alpha/beta hydrolase folding region. This superfamily comprises proteases, lipases, esterases, dehalogenases, peroxidases, epoxy hydrolases and is one of the most popular protein folds (Hotelier et al., 2004). Each carboxylesterase

enzyme core is an α/β sheet, not a barrel, with 8 strands linked by helixes. The proteins have very different substrate affinities and do not have a very large degree of resemblance in their main DNA sequences. However, esterases are believed to come from a shared ancestor because of their structural similarities and the preserved residue arrangement in the catalytic location (Oakeshott et al., 2010). The recognized structure of the α/β hydrolase fold comprises of 6 parallel α helices and eight β sheets, with the exemption of the β-2 sheet that would in few circumstances not have a parallel orientation. The folding is accountable for keeping the residues of the active site juxtaposed in the 3D form. The moieties are present on a ring and are part of a stabilized triade comprising a nucleophilic residue, an acid moiety (glutamate or aspartate), and a histidine residue. The amino acids are not contiguous in the main series but are located in 8 to 18 subunit areas of extremely preserved amino acids (Montella et al., 2012). The nucleophile prod is the most preserved characteristic of the fold, i.e., the strident turn where the nucleophile is positioned. Although all residues are required for hydrolytic action, some families miss 1 or all active site subunits in this hydrolase. These active participants of the systemic hydrolase family participate in various diverse tasks, including exterior identification and other neuro signaling progressions (Oakeshott et al., 2010). Bulky insets can be endured if relative residue locations are retained. These inserts may contain many amino acids or shape a full additional domain, such that hydrolase superfamily members have an outstanding adaptive and evolving capability, as seen with other enzymes. ESTHER (ESTerases and alpha/beta-Hydrolase Enzymes and Relatives) is a database of all existing knowledge on genes encoding proteins in this superfamily annotated. Wheelock et al. (2005) have stated that 5,237 esterase nucleotide encoding sequences, of which 318 are carboxylesterases (PF00135) have been found in the ESTHER database (Punta et al., 2012). As of August 2011, 20,711 nucleotide sequences, 3,842 of which were in the family of the carboxylesterases genes, show a rise in this class of enzymes.

The carboxylesterase gene family consists of a robust family of extremely specific proteins, some of which have significant susceptibility to the substrate, while others are not as precise or non-catalytic proteins. The esterases can produce acid and alcohol as metabolites by hydrolyzing ester bonds in a two-step reaction. The suggested catalytic process covers the nucleophilic attack of the ester bond carbonyl by the catalytic serine

hydroxyl group (Fig. 1). This hydroxyl group is improved in its nucleophilicity, and the reaction is stabilized by hydrogen bonding to catalytic histidine. The carboxyl group of the acidic member of the catalytic triad stabilizes histidine throughout the reaction. Because of the covalent linkage of the substrate's acid mobility with the serine residual, the first step liberates the alcohol metabolite, which may be acylated, carbamylated, or phosphorylated. The affinity of the histidine residue with water molecules is a vital aspect of the second stage and allows the enzyme to reoccur to vigorous conditions and discharge the acid molecule (Testa and Kramer 2007, Russell et al., 2011). This hydrolysis phase is typical between hydrolases, a nucleophilic outbreak of H_2O on the acylated enzyme and releases the carboxylic ester and the free active enzyme from the acid movement (Sogorb and Vilanova 2002). The entire family preserves two glycines, and other small moieties near the oxyanion hole necessary to stabilize the conversion among phases and sustain the substratum in place are preserved throughout the family. Esterases are distinguished from lipases by selective action on the minor and more hydrophilic substratum and the lack of a further moving helical lid to monitor entry to the catalytic site (Montella et al., 2012). Esterase substrates may be a different variety of carboxylic, thio, phospho, and other esters, but the energetics of the responses are significantly different (Fig. 2).

3. Types of esterases

3.1 Mammalian esterases

The mammalian carboxylesterases (EC 3.1.1.1) include a class of multigene whose genes are located in the endoplasmic reticulum and cytosol of a large number of tissues. These enzymes effectively catalyze the hydrolysis of chemicals containing ester and amide and medicines (including drugs) into their respective free acids. They are concerned with different detoxifying medications, environmental toxins, carcinogenic agents, or mitochondrial stimulation. The hydrolyzing of endogenous compounds, including short and long chains of acyl-glycerols, long-chain acyl-carnitine, and long-chain esters, is also catalyzed by carboxylesterases. In some animal organisms, several isozymes of hepatic microsomal carboxylesterase are implicated in the metabolic activation of some carcinogens and correlated with hepatocarcinogenesis (Satoh et al., 1998). The metabolism and toxicity of xenobiotics typical of carboxylesterases have been

assessed by Satoh (1987). Recent discoveries have contributed to a greater comprehension of the biochemical nature and the physiological importance of carboxylesterase enzymes and the genes encoding carboxylesterase (Gaustad and Løvhaug 1992).

3.2 Plant esterases

Owing to their in vitro partialities for α and β naphthyl ester substrates, isozymes A and B-esterases are characterized by electrophoresis (Carvalho et al., 2003). A β-esterase (Est-2), three α-esterases (Est-6, Est-7, and Est-8), ten αβ-esterases (Est-1, Est-3, Est-4, Est-5, Est-9, Est-10, Est-11, Est-12, Est-13, and Est-14), and discriminating organophosphate substances susceptibility for various esterases have also been shown to be impaired in the polyacrylamide gel electrophoresis (PAGE) and the leaf esterase characteristics of *A. polyneurone*. Fourteen esterase isozymes were found from cassava cultivars by PAGE analysis (Pereira et al., 2001). The amount of esterases observed in these plant species appears to be lesser than those seen in related mammalian/insect species studies (Lapenta et al., 1998). It is necessary to evaluate the exact number of α, β, and αβ esterases in plants for the functional characterization of esterases of other organisms and various tissues. These enzymes are then placed in one multigenic family of carboxyl/cholinesterases (Oakeshott et al., 1999). Sequences needed by carboxylesterase, acetylcholinesterase, and cholesterol-esterase for hydrolytic operation have been identified in quite a high degree of adaptation. The plants' carboxylesterase production was compared to the differentiation processes, and carboxylesterase inhibition was used in *Thevetia Peruviana* as a biosensor to identify selenium composites (Saritha and Kumar, 2001).

3.3 Bacterial esterases

In their traditional type, esters are converted into carboxylic acid and alcohol (Lim et al., 2010). This broad esterase nature forms the heterogeneity and versatility of this superfamily enzyme by representatives who are able to precisely capture the hydrolysis of various chemical bonds, including ester, thioester, phosphoester, amides, and epoxides (Martinez-Martinez et al., 2018; Kovacic et al., 2013). *Mycobacterium tuberculosis* (Mtb) esterases demonstrate the complex biological roles of microbial esterases with more than 40 Mtb esterases, predicted based on computational analysis.

Apparently, it is due to its various physiological mechanisms, its ability to excavate host cell lipids for energy, and its peculiar nature, which switches between slumbering and active development (Singh et al., 2010). Mtb esterases function in Mtb infectiousness and persistence, whereas microbial growth and development are affected by non-unique esterase antagonists, namely tetrahydrolipstatin (THL), alistat, and mmPOX (Goins et al., 2018). More surprisingly, esterase repression is delayed in sleep, whereas most TB medications are inactive (Delorme et al., 2012). Mtb was also moderately selective for such non-specific esterase antagonists, demonstrating that Mtb esterases are potentially new antibacterial options (Goins et al., 2018; Lehmann et al., 2018). The majority of Mtb esterases can fit in the classical α/β-hydrolases protein group with a spatial fold of 8 β-strands that create a β sheet of α-helices (Tallman et al., 2016). Nucleophilic serine produces at least 100 N-terminal amino acids in the nucleophilic site, labelled with a G-x-S-x-G motif (Larsen and Johnson, 2019). The incorporated loops and supplementary components within the lid or cap region, particularly within the lid domain, which could be interface-enabled by lipid substratum, are controlled to attain morphologically, and chemical distinguishes (Joseph et al., 2015). However, only a few 3D configurations of mtb esterases have been found, and they mostly have the typical α/β-hydrolase protein fold. This is demonstrated by the two Mtb metabolic hydrolases LipW and Rv0045c.

These two enzymes have fundamental folds identical to α/β-hydrolases but differ in their cap/lid regions and joining regions. Due to its distinctive substratum binding site, nature is directly connected with its geometry of binding pockets (McKary et al., 2016). Proline–glutamate (PE) and proline–proline–glutamate (PPE) esterases are one kind of Mtb esterase with an increasing pathogenic feature. The PE and PPE core family is a protein family called Mycobacteria-specific identified after the PE or PPE patterns present in the N-terminus protein. In infective *Mycobacterium tuberculosis* species, the family PE and PPE are expanded to 167 PE/PPE domain proteins, but only two in the quick developing and non-pathogenic mycobacterial organism Mycobacterium smegmatis (Sultana et al., 2016). Mtb immunogenicity, immune regulation, and pathogenicity are all associated with proteins in the PE and PPE region. 8 families have computer-defined α/β-hydrolase protein folding within the wider domain classes PE and PPE. The PE–PPE domain comprises 8 PE and PPE family members with a C-terminal region coding the α/β-

hydrolase protein folding and the N-terminal PE or PPE domain (Sultana et al., 2011). These family representatives have been described as esterases and simulated with a classic catalytic domain on the α/β-hydrolase protein fold. The most well-studied representative of this class, PE11 (Rv1169c or LipX), plays a part in biofilms production, triggers an immunosuppressive response and influences lipid contagiousness thresholds. The cumulative effect of PE11 on lipids of pathogenicity and biofilm formation shows that its action is biological. The effect on biofilm development varies unexpectedly regardless of whether PE11 is expressed in Mtb or *M. smegmatis* (Rastogi et al., 2017). Since several PE and PPE groups are membrane-associated or expel domain esterases, these PE-PPE region esterases could be better providers for ester drugs. Esterases, effective towards endogenous host lipids, particularly major Mtb nutritional supplies, like triacylglycerol (TAG), are the second class of Mtb esterases (Delorme et al., 2012). TAG is a strong energy source for latent or renewing Mtb, so TAG processing from the multitude or even using Mtb TAG stores can offer favorable targeted therapies for bacterial latent infections. LipY was introduced as the very first TAG esterase (Rv3097c). LipY has a PE region that controls its activity and guides LipY to the ESX-5 secretion direction (Garrett et al., 2015). LipY deficit inhibits intracellular lipid reserves from depleting and re-starting growth after drought conditions. LipY is also convoluted in Mtb immune reaction as LipY overexpression has led to increased inflammatory responses and decreased BCG *Mycobacterium bovis* vaccine efficacy in combating TB infection. LipY has a broad generic substratum but a well-defined interaction between composition and action, which could be a good fit for drug esters. Previously, Msh1, a 2nd TAG catalyzing esterase unique to host lipid hydrolysis, was reported. Since host lipids are attacked, ester hydrolysis is catalyzed outside Mtb and upregulated under a stressful environment, Msh1 could be a new drug technique and ester prodrug catalyst for the employee (Singh et al., 2017). Several recent studies have demonstrated Mtb's proteoma-wide esterase activity and monitored Mtb's esterase transfer from the infection to the whole spectrum of Mtb esterases (Lehmann et al., 2018; Tallman et al., 2016). Four latest Mtb ABPP experiments using activity-based protein profiling (ABPP) and many mechanistic-based antagonists have labeled over 80 different serine hydrolases. All these studies on proteomic ABPP suggested an association of discrete esterase subgroups

with a wide range of targeted ligands, such as THL and fluorophosphate compounds. They also found esterase, which is available even when the cells are dormant (Lehmann et al., 2018). The various subcategories of esterases found in each study could be associated with small variations in the ABPP ligand compositions, but none of the four studies have isolated LipY, despite THL and fluorophosphate antagonists reported (Tallman et al., 2016). In a recent study, THL was changed to strongly mimic mycolic acids exclusive to Mtb membrane so that the ABPP samples are more appropriate for Mtb. This THL analogue, used in combination therapy, specifically targeted 2 Mtb serine hydrolases and improved the effectiveness of the frontline therapy by over 100-fold. (Lehmann et al., 2018). Fluorogenic ester samples have illustrated the variability of intragenous and intra-strengthening expression and the rare hydrolase expression upregulated in latent germination, as an exception to proteome-wide ABPP. Synergistic fluorogenic ester scaffolds have shown the ability of mycobacterial esterases to hydrolyze small, long-chain, branched, and polar esters. Latent mycobacterial esterase behavior showed a skewed distribution of substrates with longer and more hydrophobic esters. This is important for the geometry of drug esters (Bassett et al., 2018). This preferentially triggered ester may be an outstanding time to apply variability to ester medicines. Mtb esterases have a broad spectrum of ester reactivity, making them suitable candidates for ester drugs to therapeutically locate the active agent at the Mtb infected area (Larsen and Johnson, 2019).

3.4 *Pseudomonas aeruginosa* esterases

Secretory surface esterases from an infectious agent, *Pseudomonas aeruginosa*, are among the most documented microbial esterase families (Wagner et al., 2016). Three endothelial *P. aeruginosa* esterases form overlaps in bacterial growth, persistence, and formation of biofilms, demonstrating the various aspects, architectures, and characteristics of microbial esterases (Nicolay et al., 2012). A very well characterized of these enzymes is EstA, an autotransporter epithelial esterase involved in handling biofilms, motility, and rhamnolipid formation. The N-terminal region of this automotive carrier codes the Esterase region of the EstA, which is connected by an extended kinked Helix with the C-terminal membrane span-barrel region (van den Berg, 2010). EstA is among *P. aeruginosa* basal membrane and external membrane vesicles' 30 most

important proteins. When autotransporter proteins penetrate the cellular membranes, some catabolized them; however, EstA is bound covalently to its automotive domain extending the membrane (Couto et al., 2015). EstA is a strongly sensitive esterase with a peculiar substratum feature with butyl esters for short ester substances. The biofilm construction, enhanced solution viscosity, and reduced cell thickness were all detected as *P. aeruginosa* variants inhibited the enzymatic serine in EstA. The potential of EstA to split rhamnolipid, a natural *P. aeruginosa* biosurfactant consisting of mono- and di-rhamnesis associated with 3-hydroxy acids, is considered accountable for these (Wilhelm et al., 2007). These infectivity and biofilm-derived characteristics are not shared by EstA homologs of associated nonpathogenic Pseudomonas. The reason for this phenotypic difference is attributed to EstA from *P. aeruginosa*, which has a broader substratum specificity than its homologue from *P. putida* (Leščić Ašler et al., 2017). The hydrolysis domain of EstA is also an archetype of the GSDL bacterial esterase complex, which is named after Gly-Ser-Asp-Leu (GSDL) (van den Berg, 2010). EstA corresponds to the SGNH superfamily of GSDL hydrolases. In four preserved pattern fragments, the presence of a predominant enzymatic remnant (Ser-Gly-Asn-His) defines the SGNH superfamily. The GSDL hydrolases differ from α/β-hydrolases in the realm of esterases. They miss the nucleophilic arm for their enzymatic serine and the nucleophilic serine close to the N-terminus, with a five-strand and at least four-strand arrangement (Wilhelm et al., 2011). Several pathogenic bacteria have established EstA and other SGNH hydrolases as virulence determinants, making the latter a vital esterase subdivision in antibiotic development. Many of these contagiousness GSDL esterases, as opposed to EstA from *P. aeruginosa* (Flores-Daz et al., 2016). *P. aeruginosa* also has two other esterases that serve a role in the growth, persistence, and formation of biofilms. LipC controls cell aggregation, the production of biofilms, and the development of rhamnolipids in the same manner as EstA. The mechanism of action of LipC for biofilm management could be distinctive from EstA as removal of LipC modified the phenotype of gene expression for a biofilm regulating reaction receptor protein called PhoP. A more *P. aeruginosa* esterase, LipA, may not significantly affect biofilm formation (Rosenau et al., 2010). LipA removal has a morphological effect on iron-dependent signaling and PvdS rates through an unknown mechanism of gene expression (Funken et al., 2011). The LipA

homolog in the *Acinetobacter baumannii* nosocomial pathogen is critical for developing long-chain fatty acid and improving the survival and colonization of this pathogen (Johnson et al., 2016). A large number of functions, features, and structures present in bacterial esterases, along with their capacity to target medicines and drug stimulation, are shown by these *P. aeruginosa* esterases.

3.5 Fungal esterases

Feruloyl esterases (FAE) are a multitude of enzymes that specifically catalyse estheric hydrolysis between a hydroxycinnamic acid (e.g., ferulic) and the plant poly-or oligosaccharides (Dilokpimol et al., 2018). FAEs are auxiliary enzymes that help to achieve substantial access to their action sites during the biofuel and biochemical biomass saccharification of xylanolytic and pectinolytic enzymes. There are few functional FAEs compared to more than 1000 fungal FAEs recently estimated by similarity-based genome mining that has been phylogenetically classified into various subfamilies (SFs) (Dilokpimol et al., 2016). To confirm previous genome mining and phylogenetic clusters and extensive knowledge on the behavior of fungal FAE, a selection of 27 putative and six characterized FAEs from ascomycete and basidiomycete fungi were produced heterologously and selectively in the *Pichia pastoris* and the recombinant protein biochemically characterised by Dilokpimol et al. (2018). As a result, the FAE has 20 enzymes active in pNP-ferulate and methyl hydroxycinnamate substrates covering 11 subfamilies. Many of the latest FAEs demonstrated similar behaviours to fungal FAEs previously identified.

Opening the plant's cell wall is an essential part of the bioethanol and biochemical development design method. The varied enzyme sets of complementary activity and specifics are required to complete the enzyme hydrolysis of plant biomass because of the heterogeneity and complexity of the plant cell walls (Mäkelä et al., 2014). During complete deconstruction of the cell wall, destruction of the lignin-ferulate-arabinoxylan ester bond is necessary because of the connection of ferulic acid to complexes of lignin-carbohydrate. FAE plays a crucial role in providing accessibility to lignocellulose fibers by removing ester links from plant polymers for glycoside hydrolases and polysaccharide lyases (Faulds, 2010). FAE not only acts synergistically to transform xylan to its monomers with xylanolytic enzymes but has also proven that it improves the total

saccharification of lignocellulose biomass, such as wheat straw and bagasse sugarcane when co-inculpated with cellulase and xylanase. FAE over-expression decreases cell wall amounts of esterified phenolics in the potato, and much of the time, increases sugar emissions and digestibility of the cell walls (Badhan et al., 2014). It is often used to produce transgenic plants that could modify their configuration when an enzyme (s) are activated, e.g., to minimize recalcitrance of cell walls before saccharification (Dilokpimol et al., 2016).

Glucuronoyl esterases (GEs) in the CAZy (carbohydrate-active enZYmes) database have recently been linked to the family 15 (CE15). GE is the catalyst for the cleavage of 4-O-methyl glucuronoyls and lignin alcohols of ester LCCs. These ester LCCs are especially rich in glucuronoxylane hardwoods (Mosbech et al., 2019). In 2006, glucuronoyl esterases were used to discover the cellulolytic wooden rotting mechanism of the *Schizophyllum fungus* (Bååth et al., 2018; Špániková and Biely, 2006). Since then, a variety of CE15s has been found with tiny synthetic model substrates designed to imitate the true ester of the LCC substrates of plant cell walls. Up to now, these experiments have shown that fungal CE15s are more unique to the substrate relative to bacterial CE15s. Fungal CE15s often tend to prefer bulky alcohols and 4-O-methylated substrates in the glucuronoyl mode. Practice on model substrates also demonstrates that fungal CE15s are active on polymer substrates illustrated by methyl ester glucuronoxylan. Deesterification of low molecular and high methyl esters was followed at similar rates, supposed to provide access to vast substrates through surface-exposed active sites on the CE15s (Biely et al., 2015).

4. Extremophilic esterases

4.1. Thermophilic esterases

The species adapted to live at high temperatures live between 45 °C and 122 °C and exhibit several variations on a molecular level regarding mesophilic organisms and are mostly archaea and eubacteria. The GC content of the coding regions is correlated with the weather at increased temperature; the GC content is high, which is the principal difference with the mesophilic species (Zheng and Wu, 2010). In relation to the mesophilic equivalent, membranes have a distinct structure. Many reports have shown that the membranes of thermophilic species produce higher levels of lipids that are stable at

temperatures, particularly ether lipids and esters with long acyl chains (Koga, 2012). Proteins are the major evolutionary difference in adaptation at high temperatures because high temperatures are one of the most denaturing elements. Thermophilic organisms cope with inherently resistive proteins to high temperatures and are relatively stable. Many experiments were carried out to clarify protein thermostability factors that contribute, and many factors were identified; research was carried out at the composition level of amino acids and through the contrast of the three-dimensional form with mesophilic and psychrophilic equivalents. Thermophile proteins have different stability mechanisms, as the decrease in polar residues has been observed at amino acid levels, decrease in cysteine and deamidation sites, increased loaded residues and increase in proline in loop regions; decrease in lengths of loops, increase in ion pairs, hydrophilic proteins have been identified at the structural level (De Luca and Mandrich, 2020).

In order to emphasize the discrepancies properly in amino acid composition or structure among thermophilic and mesophilic proteins, it is worth noting that a comparative study of a huge amount of proteins is only common in trending but does not lead to rigorous differences between thermophilic and mesophilic proteins. The most interesting evidence is that the growth of proteins stability, besides increasing the number of ion pairs, has been shown and increasing the number of electrostatic interactions and interaction paths that stabilize the charged residues on proteins. This is the case in Esterases of the HSL family that were established as a determining factor of thermostability (Pezzullo et al., 2013). For HSL families, the amount of proteins located at a cut-off distance of four A˚ was 16 in BREFA, four in EST 2 and eighteen in AFEST, with a cut-off of six A˚ was 22 in BREFA, two in EST2 and one in AFEST 21 in the HSL band, whereas the number of cut-offs was 3 in HSL (Mandrich and de Pascale, 2011). This study shows that the number of ion pairs does not seem to be a determining factor for thermostability; however, the evidence on the ion-pair numbers and electrostatic interactions is in accordance with the increase in thermostability through studying the pathway of contact between charged residues on the protein surface. Using a study of mutagenesis certain essential loaded residues in broad contact paths have been shown to alter the thermostability (Pezzullo et al., 2013). Such enzymes are extremely interested in biotechnological applications with the awareness regarding thermophilic enzymes in relation to function, specificity, and

stability. Probably, several lipases and esterases have now been identified, and their possible uses from food to the pharmaceutical industry are high thermal stability, greater half-life, and organic solvent constancy (Mandrich et al., 2012).

4.2. Psychrophilic esterases

The most common feature of psychrophilic species is microbes, archaea, yeasts, and algae in low-temperature regions such as high mountain regions and perennial glaciers. In this respect, psychrophilic species are undergoing adaptations designed to flourish and live best at low temperatures, sometimes at high temperatures. Another situation is Antarctic fish; the Antarctic circumpolar current that runs from the west to the east across Antarctica is evidently separated from the other animals. These fishes vary from other resistant, circulatory, anti-freeze glycoprotein or ice-binding proteins (IBPs) at the stage of the other species and have proteins adapted to low temperatures (Beers and Jayasundara, 2015). IBPs work varies from the psychrotolerant since IBPs mediate freeze-tolerance, ice adhesion, and ice re-crystalization resistance, leading to dehydration and cellular harm by forming large ice crystals. IBPs attach to a particular ice axis and induce a microcurvature to enable the ice to expand between the adsorbed IBP and the curved surface in a restricted region. Thermodynamically, it is harder to combine water molecules (Kelvin effect). Thus, water freezing temperature decreases in a non-collective manner, resulting in a hysteresis between freezing and melting temperatures (Nutt and Smith, 2008).

The molecular and functioning diversity of IBPs is important because species belonging to distinct biological kingdoms have developed separately and are hosted in various niches. The IBPs are separated into 11 distinct folds by classifying their crystal structures. Everyone shares a similar structural approach, which is consistent with the need to fold and work cold. The IBPs stabilise their formation by disulfide links and hydrogen bond networks, not through the hydrophobic center (Davies, 2014). Two major characteristics of the sites are straight and hydrophobic, free from charged residues, and repetitive sequences of amino acids, which are associated with their capacity for ice-like Water organization on some IBP surfaces (Bar Dolev et al., 2016). Low temperatures strengthen membranes, resulting in functional failure. This dilemma is solved by membrane structures. Psychrophilic species have high lipid content and have a limited acyl chain

volume, polyunsaturated fatty acids, branched methyl acids, and a high lipopolysaccharide ratio, contrasted with the mesophilic equivalent (Dhaulaniya et al., 2019). The major differences observed concerning the adjustment of psychrophilic proteins are the following: reduction in hydrophobic surface residue, decrease in aromatic interactions, decreased disulfides and salt bridges, decrease in the amount of arginine and proline, increase in glycine at the active location, increasing of enzyme dimensions, increasing of the number of polar cavities (Asn, Cies, Gln, Ser, Thr, Tyr) (Dhaulaniya et al., 2019). Both differences maintain high levels of versatility at the level of protein structures, but for this purpose, psychrophilic proteins are highly affected by thermal stability at temperatures of around 37 °C. To this era, a number of psychrophilic esterases have been insulated and mostly investigated to explain the cold tolerance molecular determinants, everywhere the substratum is sensitive to temperatures such as fruit, organic synthesis, animal feed, textiles, detergents, and beverages (Ramnath et al., 2017).

4.3. Halophiles and alkalophiles

The species are mostly archaea and bacteria suited to living at elevated salt levels (maximum 5 M NaCl). They are capable of maintaining the osmotic equilibrium of salt accumulated at isotonic levels. Halophilic proteins mostly adjust at protein surface levels to avoid precipitation by growing the number of negative wastes, but this adaptation often provides consistency with low water content. Numerous esterases have been isolated and categorized from halophilic species (De Luca and Mandrich, 2020). Alkalophile species may survive in high or low pH environments. They can preserve internal pH close to neutrality by proton pumps, and thus, besides those in periplasm space, the proteins do not require adaptation. Few lipases can be separated for high-pH adjustment and used in detergent preparation for fat hydrolysis, where high pH values are normally used (De Luca and Mandrich, 2020).

5. Exploring novel esterases by genomic and metagenomic tools

The sequence automatic processes and shotgun cloning have launched several genome projects that include a great deal of genetic data. To date, the Genome Atlas Database has included 1078 bacterial genomes and 82 Archaea (Hallin and Ussery, 2004). Selected results reveal many enzymes, which were subsequently cloned, overexpressed,

and purified for biochemical characterization by genome mining for new genes through homology with identified lipase and esterases. Thus a few lipolytic enzymes were cloned and expressed in mesophilic hosts from *Thermus thermophilus* HB27 whose genome is fully sequenced and accessible publicly (Henne et al., 2004). Extremely thermal stability and a very high behavior at mesophilic temperatures were obtained with a significant proprietary reciprocal esterase, a significant fact of its thermophilic nature (López-laópez et al. 2010).

At this time, the origins of new enzymes are theoretically restricted to a small fraction of the overall microbiome, which accounts for less than 1% of the actual microbial communities in many ecosystems. With the study of metagenome, genome pool, the Metagenomics Era represented a step ahead (López-López et al., 2014). Current technology in molecular biology allows complete environmental DNA libraries to be created, including the genomes of non-cultural species, which open a new window into a large area of possibly newly unknown enzymes. The increasing amount of industrial-interest enzymes found in metagenomic studies is likely to surpass conventional enzymes quite shortly. It is noteworthy that recent biocatalysts in metagenomic DNA libraries concentrate primarily on producing a small category of enzymes, which includes esterases (Streit and Schmitz, 2004).

With the advancement of next-generation sequencing technology and emerging bioinformatics methods for broad study and classification of metagenomic databases, several metagenomic sequencing projects result in a holistic view of microbial communities' taxonomic and ecological diversity (Simon and Daniel, 2011). According to GOLD (Genomes Online Database), 340 sequenced metagenomes are available, 197 from natural environments (mostly aquatic), 114 from associated host environments, and 29 from engineered environments, respectively (Singh et al., 2014). New enzymes are identified by analyzing the accessible metagenomic evidence for enzymes like existing esterases in a sequence-based metagenomic method. A PCR-based approach with degenerate primers is another popular technique, constructed according to the conserved regions of the previously identified enzyme groups. One drawback is that this technique only detects enzymes related to previously identified groups (Bell et al., 2002). Alternatively, a practical screening may be performed to detect clones with lipolytic

activity. The success of these screenings is based on the interaction of cloned genes with the heterologous host, *Escherichia coli's* transcription and translation machinery. In addition, the need for some chaperones to correctly fold the enzyme or the toxicity of the enzyme to host cells can impair the expression of a certain enzyme. There have been reports that a practical screening of *E. coli* as host only recovers a subset of enzymes with the required behavior in a metagenomic library, about 40% (López-López et al., 2014).

The utility for addressing the host compatibility barrier of Broad-Host Range vectors was evaluated. One of the latest studies shows that six different proteobacteria are efficient hosts of the same metagenomic cosmid library to retrieve various positive clones from each host (Craig et al., 2010). More recently, a cosmic vector was developed by Lussier et al. (2011) that offers a two-way stretch: multi-host (*E. coli* and *streptomyces lividans*) expression and T7 RNA polymerases transcription, with high activity, very long mRNAs and very poor termination by unrelated transcripts, which may potentially increase the expression of alien genes in large insert libraries, A special cosmid vector has also been created to express thermophile metagenomic books, which enable for the building of a library in *E. coli* and subsequently for expression and screening in *Thermus thermophilus* (Angelov et al., 2009).

The benefit of the functionally guided method is the capacity to identify whole new gene or enzyme groups without any similitude with identified esterases. Wang et al. (2010) created a metagenomic screening variant called the "truncated metagenomic gene-specific PCR" to circumvent the shortcomings of the traditional sequence-based method. A metagenome-derived lipolytic enzyme is the starting point of this process, used for the development of primers degenerated by DNA molding and to analyze sample diversity. A diversified library of lipolytic enzymes of varying specificity has been acquired. The most common screening procedure used with tributyrin agar plates to detect positive clones that exhibit the required lipolytic behavior indicates simple hydrolysis of the substratum. Screenings have also been used for metagenomic libraries to detect true lipases with long substrates not hydrolyzed by esterases (for example, emulsified triolein, tricapryline or olive oil) in the presence of fluorescent rhodamine B coloration. In this scenario, orange

fluorescent halos occur at 350 nm, when UV-irradiated, around colonies containing lipase (López-López et al., 2014).

In a soft-blue-Agar-Oberlay (detection of yellow halos) and agar plates in the presence of CaCl2 (detection of halos produced by released fatty acid salts), metagenomic libraries are less commonly screened utilizing alternate substrates such as α-naphthyl acetate (Okamura et al., 2010). One use of these enzymes is the organic synthesis of optically pure substances. Therefore, new approaches for enantioselectivity screening are essential. Scientists have established a sensitive, economic, and scalable approach focused on using sodium fluorescein as an indicator in this respect. This test enables unique esters from different substrates, not only from chiral carboxylic acids but also from chiral alcohol, to be classified in a 96-well plate format (Wang et al., 2009).

The insert size is another essential matter for metagenomic screening performance. As plasmids are used, they are cloned in comparatively short metagenomic sequences (< 10kb) and more clones than in a metagenomic library of cosmid or cosmic vectors, where the insert sizes are 40kb respectively and 25-35kb. Moreover, massive gene and operon clusters cannot be retrieved with small inserts. The use of plasmids that enable bidirectional transcription and facilitate a multi-cloning site on both sides are an alternative for decreasing the plasmid library size required to detect positive clones. The lipolytic activity was investigated using this kind of plasmid, producing large frequencies of positive clones similar to those obtained in cosmid libraries (Lämmle et al. 2007).

The frequency of clones expressing the necessary behavior is normally lower than 0.1% for functional metagenomics (carrying a properly expressed lipase enzyme coding gene). A culture enrichment phase before the library's building will increase this benefit at the cost of a large drop in microbial diversity. This technique encourages the development of target enzyme-hosted microorganisms by using some physical-chemical pressure substrates or resistances. For instance, the growth of the thermophilic enzymes harboured by high culture temperatures. As such, in a sequence, Fed-Batch Reactor at 50-70 °C over three months of cultivation, seven novel alkaline and thermophilic enzymes have been discovered by cultivation enrichment (Meilleur et al. 2009; Côété and Shareck, 2010).

6. Immobilization of esterases for improving biocatalytic properties

The best method for reducing process costs could be enzyme immobilization since it would allow the retrieval of enzymes (Almulaiky et al., 2020; Gan et al., 2020; Qamar et al., 2020; Bilal et al., 2020). Immobilized enzymes on the exterior of usable carriers take numerous benefits above soluble enzymes, comprising recycle and reuse options, improved stability and catalytic activity, and fast functioning (Chaubey et al., 2006; Aslam et al., 2021; Bilal and Iqbal, 2019a,b). Several strategies for immobilizing proteins are accessible in solid substrates and classified into four major groups: adsorption, entrapment, cross-linking, and covalent binding utilizing profitable resources (Asgher et al., 2017; Adeel et al., 2018; Bilal and Iqbal, 2019c; Ren et al., 2019, 2020). There are advantages and drawbacks to each approach. Enzyme confirmation is mainly preserved in physical adsorption because adsorption is primarily accomplished by either electrostatic interference or van der Waals' force (Sassolas et al., 2012). However, these linkages are comparatively small, and carriers' enzyme leakage can occur during the operating phase. Sol-gel trapping is commonly used due to its moderate reaction conditions. Alternative techniques for the formulation of sol-gel for the production of nanostructures and immobilizing enzymes have been suggested. However, there are certain drawbacks, such as enzyme outflow. Cross-connecting and covalent attachment benefit from an enzyme's deep irreversible connection to the supportive mediator that can shield the enzyme from the leak. A disadvantage of this tight binding is the chance of enzyme activity loss (Kloskowski et al., 2010). A number of carriers (biopolymers, nanocomposites, etc.) have been used to immobilize enzymes in addition to the various immobilization approaches (Bilal et al., 2018; Bilal et al., 2019a,b). Many studies have confirmed the immobilization of esterases on stable supports. Fan et al. (2017) reported the immobilization of cold-adapted pyrethroid-hydrolyzing esterase on a matrix of mesoporous silica SBA-15. Likewise, Grajales-Hernández et al. (2020) and Chong et al. (2019) reported the immobilization of feruloyl esterase on epoxy-activated carriers and mesoporous silica particles, respectively (Fig. 3).

7. Biotechnological applications of esterases

7.1 Biomass degradation

Biomass degradation includes the synergistic effect of a number of cellulolytic, xylanolytic and pectinolytic esterases. Because of its capacity to hydrolyze ester bonds between

cellulose residuals and phenolic compounds, Cinnamoyl Esterases are active in a disorganization network as' helper 'enzymes, making it easy for hydrolases to reach the mainstay of cell wall polymers. This preparation is beneficial in a range of uses as described underneath (Benoit et al., 2007). The main challenge in obtaining fibers of good quality is the removal of lignin, which is anatomically rooted in the pulp network and accountable for the black color of the pulp (Bilal et al., 2017; Asgher et al., 2018; Rashid et al., 2021). In order to bleach and eliminate lignin, the Kraft method utilizes chemical therapies of chlorine compounds, which contain high dioxin and chlorolignins, which are contaminating complexes. The increased biologic conversion characteristics provide an intriguing substitute for organic bleaching. Hemicellulases and oxidoreductases such as xylanases and laccases are used in pulp bleaching to reduce chlorine intake and improve the final luminosity of pulp (Mayer and Staples 2002). In combination with xylanase and laccase, *A. niger* FaeA led to effective delignification of the pulp of wheat straw. *A. niger* FaeA has also been used with oilseed flax straw in a completely free chlorine phase, resulting in a very small amount of kappas (directly commensurate with lignin content), a good influence on pulp lightness and phenolic compounds of interest (Tapin et al., 2006). The development of fuel ethanol from sustainable lignocellulosic materials is another non-food use of feruloyl esterases. In conjunction with xylanases and laccases to produce bioethanol, *A. niger* FaeA was also used to convert lignocellulosic biomass to fermentable sugar. The effectiveness of the enzyme therapy was assessed in the saccharification step by calculating sugar yield with the best results with a FaeA and Xylanase combination (Tabka et al., 2006). Phenolics, such as ferulic, p-coumaric, caffeic, and sinapic acids, are released from the plant wall through Feruloyl esterases. These phenolic compounds in the kingdom of plants are commonly dispersed and increasingly being looked upon in fruit, hygiene, cosmetics, and drug applications. Ferulic acid can serve various biological roles, including UV absorbing, antioxidant and anti-inflammatory functions. It is one of beer's main antioxidant components, although its production during storage is triggered by orange juice. The antioxidant function of phenolic acids is mostly attributed to their chemical composition and the aromatic ring of hydroxy classes. There is also an increase in antioxidant efficiency of two hydroxy groups on cafeic acid relative to one on ferulic acid (Benoit et al., 2007).

7.2 Food industry, flavorings, and alcoholic industry

Esterases may promote the division of esters into acid and alcohol in the aqueous solution. Moreover, esterases hydrolyze short-chain acylglycerols, instead of long-chain ones and are also distinct from lipases. Esterases show a leading part in the foodstuff and alcohol industry, where they are often used to modify oil and fat in different fruit juices and create fragrances and flavours (Raveendran et al., 2018). The esteric bond between ferolic acid and various polysaccharides in plant cell walls is breached by Feroloyl esterases, an essential category of enzymes from the esterase family. As feruloyl esterases, lignocellulosic biomass hydrolyses are unavoidable in waste management (Faulds, 2010).

In a metagenomic library from the Cow Rumen Microbial Community, Cheng et al. (2012) examined the behavior of feruloyl esterase and determined that feruloyl esterase, which may release acid from wheat straw, could be protease resistance. Due to its strong pH and thermal stability, and protease tolerance, this specific esterase has great commercial applications. Diverse methyl or ethyl esters of short-chain fatty acids provide fruity flavor in cheese manufacture. Ethyl esters and thioesters are known to be generated by bacteria. The new thermostable esterase from the highly thermotolerant *Bacillus licheniformis* heterologously expressed in E. coli was generated for the development of short-chain flavor esters by Alvarez-Macarie and Baratti (2000). Alvarez-Macarie and Baratti recorded Feruloyle esterase, the precursor to vanillin, the flavour compound found in food and drink, as one of the key enzymes of ferulic acid biosynthesis. Microbial synthesis of ferulylesterase has been confirmed by several researchers (Raveendran et al., 2018).

FAEs have surprisingly been utilized both to remove odors and to improve the fragrance of many seasonings. FAEs have often been used to enhance the aroma. Flavour and smell are essential to performance in the luxurious fermented seasonings, particularly in the Japanese rice wines and in the alcoholic beverage industry, sake, and mirin. Ferulic acid and its byproducts, including 4-vinyl guaiacarol, vanillic acid, and vanillin are the main aroma components of these drugs. FAEs may be used as a koji generating FAE or as an intermediate along with xylanases and cellulases in the saccharification phase to enhance

the discharge of fertilizers from rice and other cereal grain cell walls and turn them in fermentation and ageing to aromatic derived products (Kanauchi, 2012).

7.3 Chemical preparation

Ferulic acid and other hydroxycinnamic acids are phenolic phytochemicals commonly used in their special and effective properties like the antioxidant, sunblock, depigmentation agent, precursor for flavoured compounds, etc., in food and cosmetic industries. Ferulic acids and other hydroxycinnamic acids may be employed as vitamin C and E carriers, which double their skin photoprotection with increased lipophilicity, enhancing penetration into the stratum corneum (Lin et al., 2005). They also exhibit positive therapeutic and health properties such as antimicrobial, non-inflammatory, anti-diabetic, anti-thrombosis agents, anti-cancer agents, and lowering of cholesterol. While commercial ferulic acid (as β-oryzanol) is mainly made from rice oil, modern processes concentrate on ferulic acid production by FAEs combined with other bio-refinery hydrolases (Dilokpimol et al., 2016).

In addition to hydrolysis, the ester-linked hydroxycinnamic acid syntheses can be used to produce goods with different chemical and biological properties by exchanging the organic ester (donor) group for the organic alcohol group (acceptor). *Arabinosis* and *arabinobiosis* accepters were the first FAE transesterification report on *Sporotrichum thermophilia* (StFaeC) (Topakas et al., 2005). The biochemical properties of feruloylated arabinose and oligosaccharides are both hydrophobic ferulic and hydrophilic oligosaccharide moieties. This includes antioxidant activity, probiotic effects, or anti-glycation inhibitions of interest in the food, pharmaceutical, and cosmetic industries with numerous applications (Ou et al., 2016). The benefit of hydrolase or transmission using transesterification is that their flexible molecules range from various sugars, aliphatic and aromatic alcohols to glycerol and propolis. In the latter case, FAEs can also be used to impoverish propolis allergy by removing caffeic acid esters under hydrolytic conditions (Dilokpimol et al., 2016).

7.4 Feed industry

The basic criteria for animal feed are fiber digestibility. Failed ingestion can impede animal development and trigger immunological stress, leading to feeding conversion in animals and thus limiting farmers' profitability. Ferulic and hydroxycinnamic acids may promote

animal health by themselves. However, feruloylation is a major inhibitor of the ruminant digestive system on plant cell walls, chiefly with an increased drilling diet (Dilokpimol et al., 2016). The addition of FAEs or FAE enzyme cocktails may enhance the access of major enzymes that degrade the chain, leading to increased fiber digestion and bioavailability of phytonutrients, accelerating animal development, and reducing immune stress (Jayaraman et al., 2015).

7.5 Biosensor development

While manufacturing cholesterol-dependent biosensors, cholesterol esterase was immobilized on polyaniline films combined with cholesterol oxidase and peroxidase and was used as sensing agents for cholesterol estimations and enhanced biosensor electrodes shelf durability (Singh et al., 2006). In the clinical diagnosis and prevention of a variety of clinical disorders such as hypertension, arteriosclerosis disorders, tcerebral thrombosis, and coronary heart diseases, the estimation of metabolites such as Glucose, Urea, and cholesterol in the blood sample is essential (Kohli and Gupta, 2016). Recently, extremely responsive fluorogenic esterase probes, obtained from the far-red fluorophore 7-hydroxy-9H-(1,3 dichloro-9,9-dimethylacridin-2-one) (DDAO), were used for the detection of low PIC levels at various stages of tuberculosis infection in mycobacterial lysates (Tallman and Beatty, 2015). Classifying Mycobacterium tuberculosis esterases in the disease has been challenging since most inclusion bodies develop in heterologous hosts. Esterase with ferrocene capped gold nanoparticles was used in blood samples to analyze cholesterol in a recent study (Davis-Lorton, 2015).

7.6 Chiral drug synthesis

Esterases are used principally to manufacture optically pure substances and pharmaceutical products, such as antibiotics and anti-inflammatory medicinal products. Esterase developed chiral medicines, including anti-inflammatory medicines used in the pharmaceutical industry as an agent to destroy pain. An esterase from *Trichosporon brassicae* has been widely used to produce optically pure (S)-and/or (R)-ketoprofen [2-(3 benzoylphenyl) propionic acid], which is very useful in reducing inflammation and pain caused by asthma, sunburn, menstruation, and fever (Kohli and Gupta, 2016). Stereospecific transformations in taxol-semi-syntheses, i.e., thromboxane-A2-antagonist, acetylcholine esterase inhibitors, anticholesterol drugs, have been identified in the

synthesis of pharmaceutical intermediates. An esterase of *Pseudomonas stutzeri* A1501, with uniquely stereospecific characteristics, has been described for its use in industrial synthesis (Lehmann et al., 2014).

7.7 Anti-tumor and anti-cancer

Different lethal cancers have been identified, and one of them is lung cancer. There is a lack of precision and efficacy in recent chemotherapeutic methods for lung cancer. The nanotherapeutic medicinal products β-lapachone (β-lap) have been transformed into porcine liver Esterase using bioconsistent and biodegradable poly (ethyleneglycol)-b-poly (D, L-26 lactic acid) (PEG-b-PLA) micellulose β-lap-dC3 and by 28-dC6 (PLE). Antitumor efficacy and long-term survival with cytotoxicity assays in A549 and H596 lung cancer cells were demonstrated in the β-lapachone product (Ma et al., 2015). The anti-tumor effect of carboxylesterase (CE) expressive NSCs has been shown to treat primary lung cancer or metastatic lung cancer in the brain in a neural stem cell (NSC) dependent enzyme/prodrug therapy (NDEPT) (Yi et al., 2014). The development of A549 human non-small cell lung adenocarcinomas in vitro and in vivo, thereby supplying therapeutic genes to brain tumours has been used as an important therapy for brain metastases from lung cancer (NSC) expressing rabbit carboxylesterase (F3. CE) (Hong et al., 2013).

7.8 Neuropathy

Neuropathy is a nerve condition that may adversely influence the gland or organ's feeling, reaction, or activity. NTE (Neuropathy Target Esterase) is a membrane-bound protein present in vertebrate neurons that plays a key role in chemically induced and naturally occurring neurological disorders (Kropp et al., 2004). During the testing of possible organophosphorus neurotoxicants (paraoxon, malaoxon, chlorpyrifos-oxon, dichlorovos, and trichlorfon) on neuroblastoma cell lines (human SHSY5Y and murine NB41A3), it was discovered that organophosphorous compounds inhibit target esterases acetylcholinesterase (AChE) and NTE, resulting in acute and delayed neurotoxicity. The function of NTE in neurodegeneration was demonstrated experimentally in NTE knockout mice produced by cre-loxP site-specific recombination, which revealed that NTE deficiency resulted in neuronal vacuolation and extensive membrane deformities in hippocampal and thalamic neurons (Akassoglou et al., 2004). Loss of NTE phospholipase activity and accumulation of phosphatidylcholine attributable to organophosphorus

mediated delayed neuropathy (OPIDN) resulted in endoplasmic reticulum dysfunction and axonal transport hindrance, according to studies in mammalian cell lines and yeasts (Glynn, 2007). This is illustrated by the pathway in which NTE deacetylates phosphatidylcholine (PtdCho) at the cytoplasmic face of the endoplasmic reticulum membrane to shape soluble products such as free fatty acids (FFA) and glycerophosphocholine (GroPCho), but organophosphate inhibition results in OPIDN (Zaccheo et al., 2004). The function of NTE in metabolism and pathophysiology was recently reviewed. NTE-mediated synthesis of glycerophosphocholine, an abundant organic osmolyte in renal medullary cells, protects renal medullary cells from elevated interstitial concentrations of NaCl and urea. The function of NTE in controlling the cytotoxic aggregation of lysophospholipid in mammalian membranes and maintaining lipid bilayer fluidity has been established. The influence of the hydrolysis of 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine (p-lysoPC) by the catalytic domain of NTE on different assisted bilayer membranes (sBLMs) formulations were studied using the fluorescence recovery after pattern photobleaching (FRAPP). It was concluded that the fluidity of sBLMsm reconstituted on silica decreased significantly (Greiner et al., 2010).

7.9 Treatment of hereditary angioedema and hypercholesterolemia

Hereditary angioedema (HAE) is an autosomal dominant condition, marked by outbreaks of debilitating fluid accumulation, which normally affect bowel movements, face and upper airways, truncation of the neck, genitals, and limbs. The C1 esterase inhibitor in HAE (C1-INH) is deficient with no prevalence disparities depending on sex or race (Bork, 2014). HAE management can provide care for acute attacks or long-term preventive prophylaxis for HAE attacks (Pham et al., 2014). A trial of daily intravenous infusion of human-plasma-derived C1-esterase Inhibitor (pdC1-INH) concentrate in the HAE patient who contained hypovolemic shock, asthma, extreme nosocomial pneumonia sepsis, renal and respiratory failure was tested for its clinical status, particularly renal function (Cowan et al., 2001). Therefore, human plasma-derived esterase C1 (pdC1-INH) inhibitors are recommended to control HAE prevention and HAE occurrences in medically treated patients. Hypercholesterol is distinguished by extremely elevated serum cholesterol levels and is a known risk factor for atherosclerosis and CHD in humans (Heidrich et al.,

2004). A study noticed that targeting cholesterol esterase inhibitors may be helpful therapies for limiting cholesterol absorption (Ellidag et al., 2014).

7.10 Gene therapy and diagnostics

Gene therapy requires substituting a functional gene for a dysfunctional gene in the body. This may be achieved by separating the cells from the body by utilizing techniques of genetic technology to alter faulty sequences in the DNA. A gene supply vector for the hydrolytic degradation of organophosphate was engineered to increase serum PON1 paraoxonase/ararylesterase enzymes before entering the cervix, causing toxicity (Kohli and Gupta, 2016). Multiple myeloma is a cancer of plasma cells, representing 1% of neoplastic and 13% of haematological disorders in the USA. In patients with myeloma multiple, arylesterase function in the controls and patients with elevated oxidative stress was found to be substantially lower (Howell et al., 2014). The degradation of cocaine in rats and defense against convulsive and fatal effects of cocaine were reported to avoid harmful cocaine effects on the central nervous system (Aïzoun et al., 2013). The function of esterases in implementing malaria control strategies has also been established, which assists insecticide resistance to bendiocarb in *Anopheles gambiae* Tanguieta. Leukocyte esterase has recently been suggested in the synovial fluid as a proxy for periprosthetic joint infection (Tischler et al., 2014). Table 1 and Fig. 4 portrays the sources and biotechnological applications of esterases.

9. Conclusion and perspectives

Esterases are widely used in the food, detergent, pharmaceutical, and paper industries. In terms of environmental friendliness, enzymatic hydrolysis and enzyme-dependent processes now favor effective process management, high output, low processing costs, and system protection. Esterases are also used to cure cancer by diagnosing neurodegeneration, and other debilitating conditions such as inherited angioedema and hypercholesterolemia. As a result of their function in producing β-lap prodrug-nanotherapeutics, newer enzymes are being used in medicine to treat neural stem cell cancer, chiral drug-synthesis, and gene therapy. Microbial esterases have been better utilized in microbial fermentation for recombining and protein technology, but animal and plant esterases are frequently used. Esterases are extremely powerful ecological enzymes that are frequently used in xenobiotic and bioremediation detoxification. The

extremophilic esterases give an advantage over their utilization in extreme environments. Esterases have far less literature than lipases, making the study of these enzymes quite necessary and highly useful as a future field of study in this area. Metagenomic and immobilization techniques prove beneficial in the isolation and utilization of these enzymes. Since there is a limited attention of researchers in these areas, it is necessary to give attention to the utilization of metagenomic and immobilization techniques to take advantage of these enzymes at broader scale.

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Conflict of interests

The author(s) declare no conflicting interests.

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List of Figures

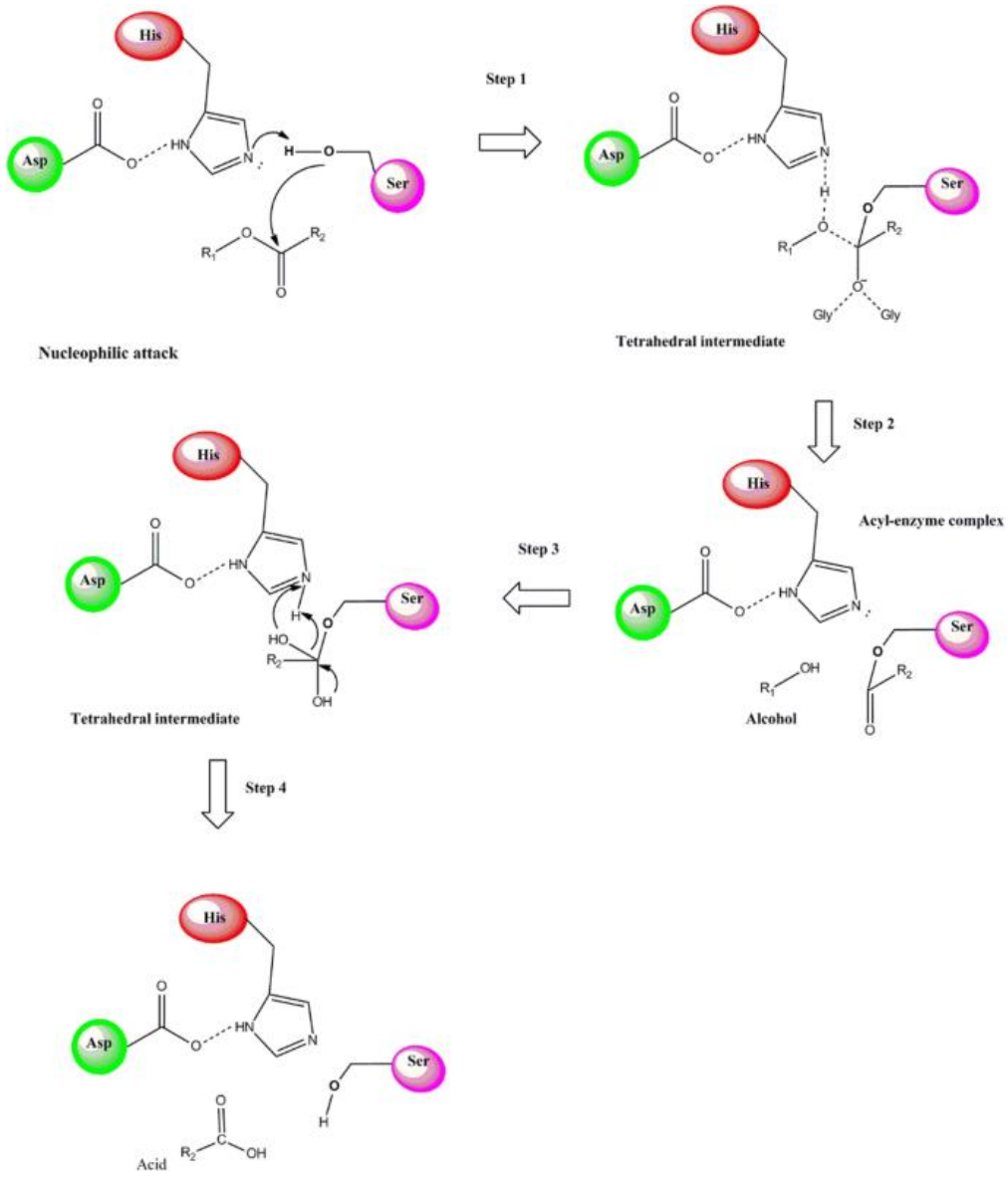


Fig. 1 Mechanism of action of esterases (Reproduced from Barzkar et al., 2021 with permission; Copyright © 2021, The Author(s), under exclusive licence to Springer Science Business Media, LLC part of Springer Nature: License Number 5156570657915

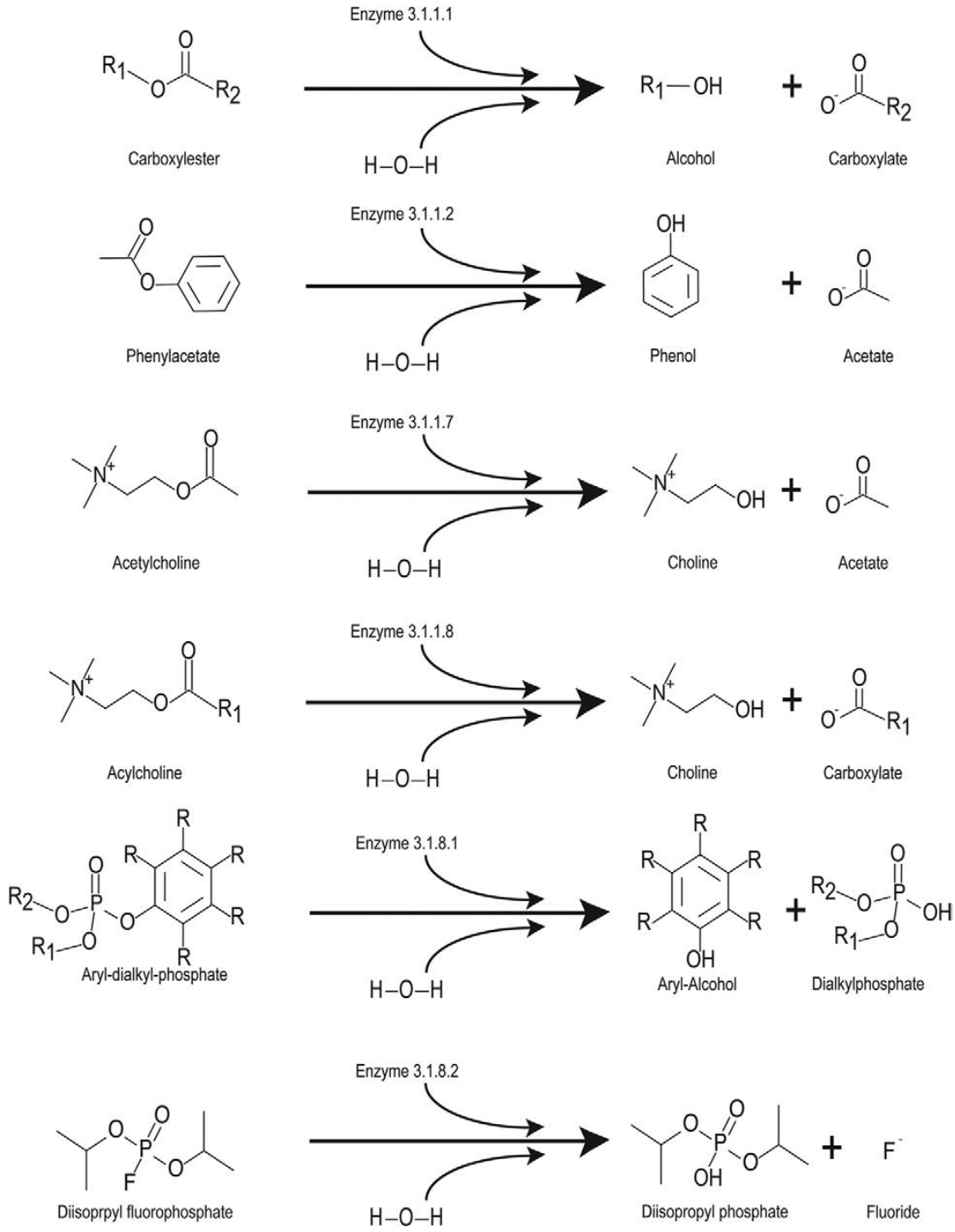


Fig. 2 Esterase mediated hydrolysis of different substances. Reproduced from Montella et al., 2012 with permission; This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

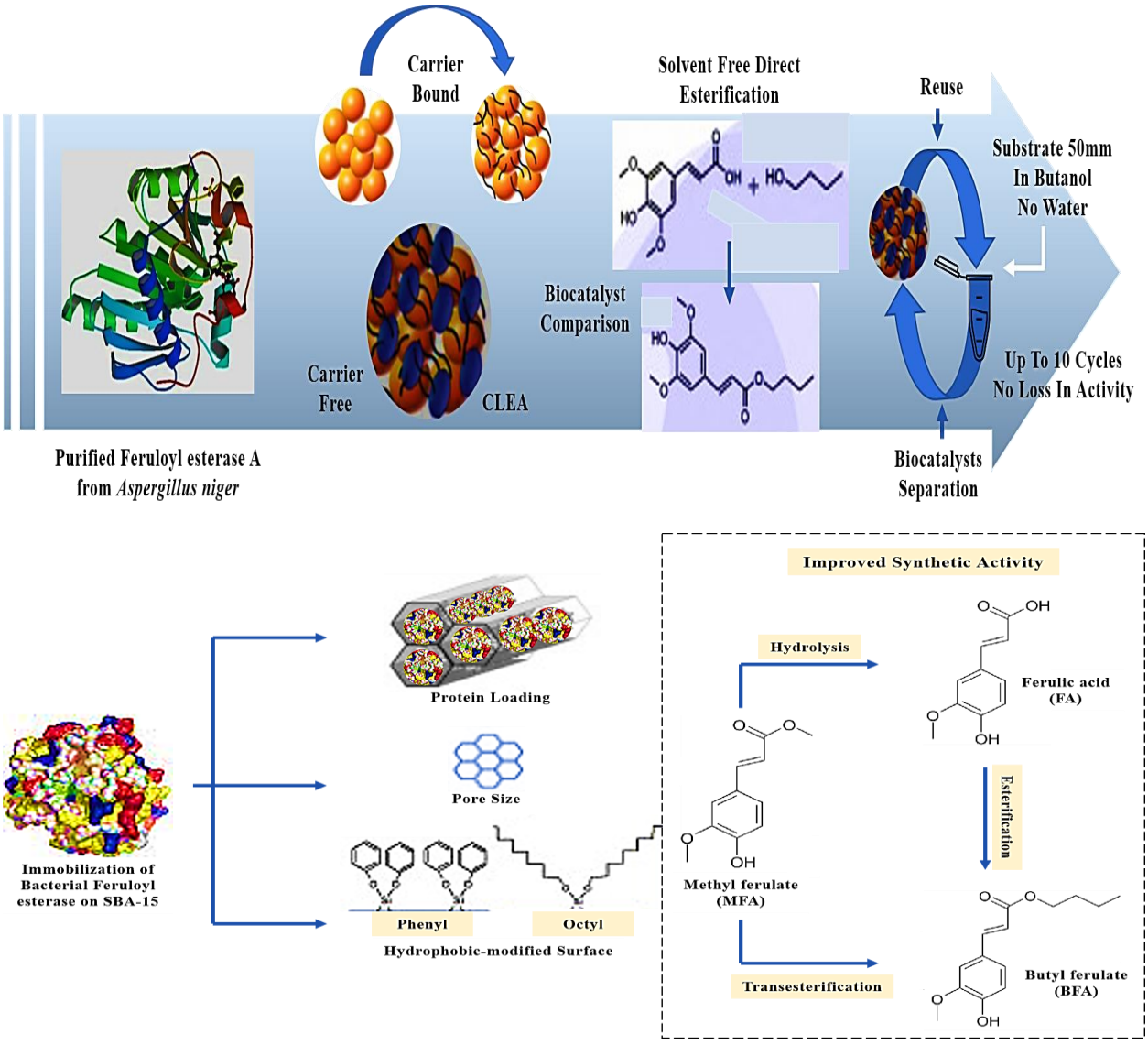
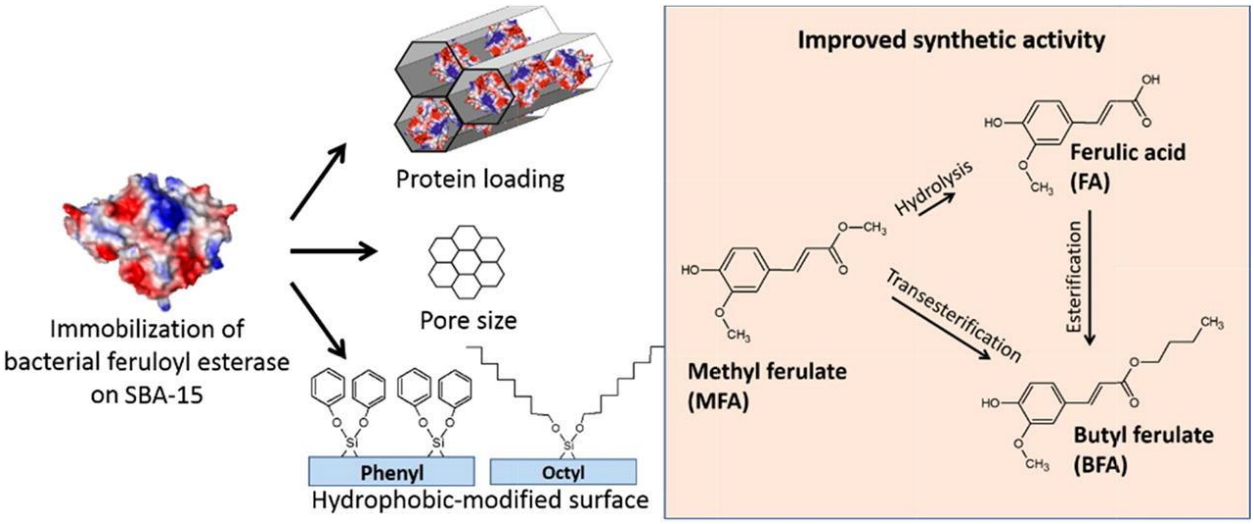


Fig. 3 Immobilization of feruloyl esterase on (a) epoxy-activated carriers and (b) mesoporous silica.



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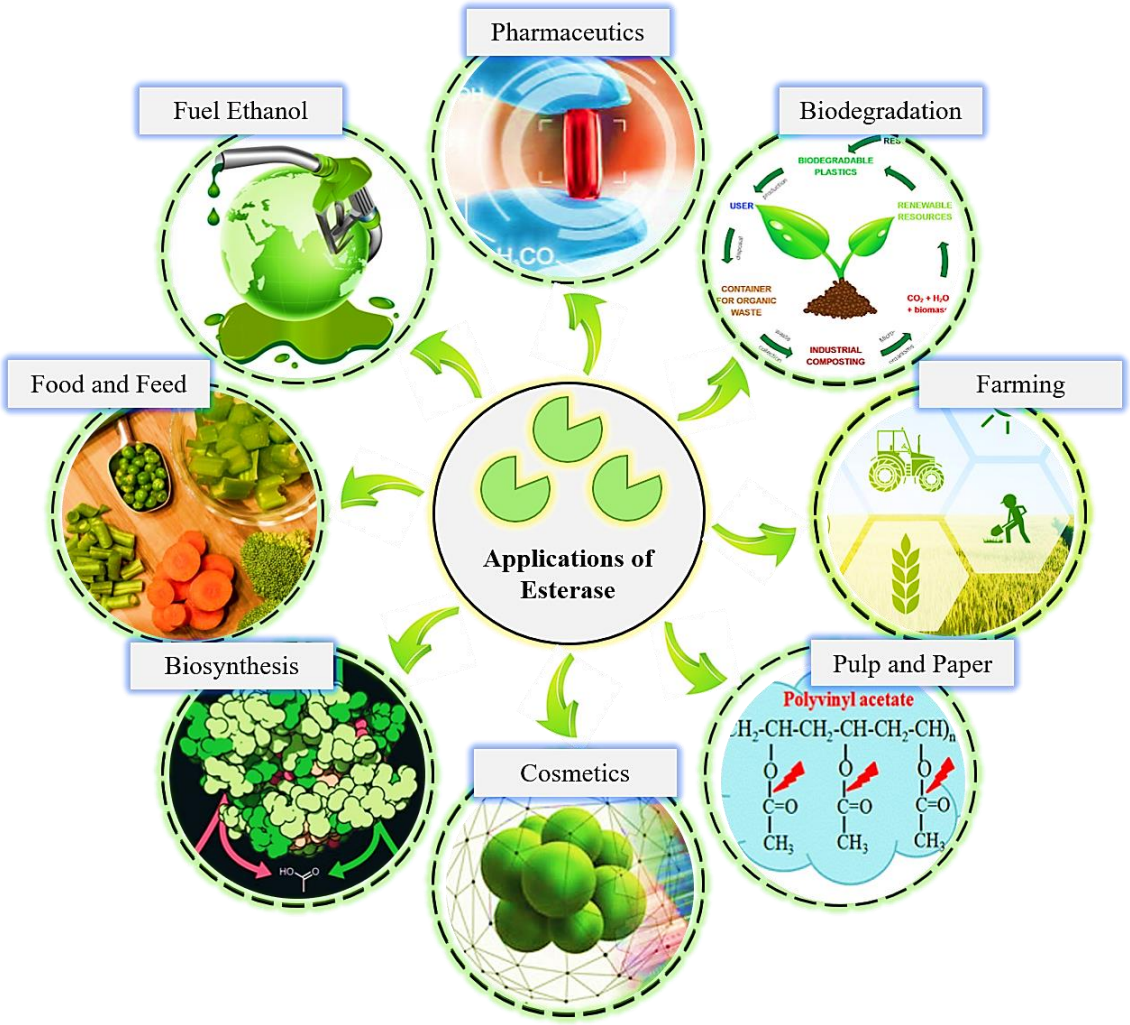

Fig. 4 Applications of estrases in different industrial sectors.

1342 **Table 1** Sources and biotechnological applications of esterases.

Enzyme	Source	Application	References
Esterase from *Trichosporon brassicae*	Fungi	Biomedicine	Kohli and Gupta (2016)
Esterase from *Pseudomonas stutzeri* A1501	Bacteria	Biomedicine	Lehmann et al., (2014)
Neuropathy Target Esterase (NTE)	Mammal	Neuropathy	Akassoglou et al., (2004)
Porcine liver Esterase	Mammal	Anticancer	Ma et al., (2015)
Carboxyl esterase	Mammal	Antitumor	Hong et al., (2013)
Cholesterol esterase	Bacteria, Mammal, *Pseudomonas aeruginosa*	Biosenssor	Singh et al., (2006)
Arylesterase	Mammal, Bacteria	Antioxidant	Howell et al., (2014)
Leukocyte esterase	Mammal	periprothesis joint infection	Tischler et al., (2014)
cellulolytic, xylanolytic, pectinolytic esterases, Laccases	Plants, Bacteria	Biomass degradation	Tabka et al., (2006); Mayer and Staples (2002)
Feruloyl esterases	Fungi, Bacteria	Biofuel, antioxidant, anti-inflammatory, UV protector, Flavoring compounds, animal feed	Tabka et al., (2006); Dilokpimol et al., (2016); Jayaraman et al., (2015); Kanauchi, (2012)

1343