Microbial carboxyl esterases: classification, properties and application in biocatalysis

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Abstract

Esterases (EC 3.1.1.x) represent a diverse group of hydrolases catalyzing the cleavage and formation of ester bonds and are widely distributed in animals, plants and microorganisms. Beside lipases, a considerable number of microbial carboxyl esterases have also been discovered and overexpressed. This review summarizes their properties and classification. Special emphasis is given on their application in organic synthesis for the resolution of racemates and prostereogenic compounds. In addition, recent results for altering their properties by directed evolution are presented. © 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Carboxyl esterase; Microbial; Biocatalysis; Organic synthesis; Directed evolution

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1. Introduction

Esterases (EC 3.1.1.x) represent a diverse group of hydrolases catalyzing the cleavage and formation of ester bonds. They are widely distributed in animals, plants and microorganisms. Many of them show a wide substrate tolerance which led to the assumption that they have evolved to enable access to carbon sources or to be involved in catabolic pathways. Moreover, esterases also show high regio- and stereospecificity, which makes them attractive biocatalysts for the production of optically pure compounds in fine-chemicals synthesis [1–6]. The interest in these enzymes also resides in the fact that they do not require cofactors, are usually rather stable and are even active in organic solvents.
Two major classes of hydrolylases are of utmost importance: lipases (EC 3.1.1.1, triacylglycerol hydrolases) and ‘true’ esterases (EC 3.1.1.3, carboxyl ester hydrolases). The three-dimensional (3D) structures of both enzymes show the characteristic $\alpha/\beta$-hydrolase fold [7] – a definite order of $\alpha$-helices and $\beta$-sheets – which is also found in haloperoxidases and epoxide hydrolases (Fig. 1). The catalytic triad is composed of Ser-Asp-His (Glu instead of Asp for some lipases) and usually also a consensus sequence (Gly-x-Ser-x-Gly) is found around the active site serine. More recently, esterases have been identified containing a Gly-x-x-Leu motif [8] as well as enzymes showing high homology to class C $\beta$-lactamases [9]. The mechanism for ester hydrolysis or formation is essentially the same for lipases and esterases and is composed of four steps: First, the substrate is bound to the active serine, yielding a tetrahedral intermediate stabilized by the catalytic His and Asp residues. Next, the alcohol is released and an acyl-enzyme complex is formed. Attack of a nucleophile (water in hydrolysis, alcohol or ester in (trans)-esterification) forms again a tetrahedral intermediate, which after resolution yields the product (an acid or an ester) and free enzyme [1].

Lipases can be distinguished from esterases by the phenomenon of interfacial activation, which was only observed for lipases. Whereas esterases obey classical Michaelis–Menten kinetics, lipases need a minimum substrate concentration before high activity is observed. Structure elucidation revealed that this interfacial activation is due to a hydrophobic domain (lid) covering the active site of lipases – only in the presence of a minimum substrate concentration, i.e. a triglyceride phase or a hydrophobic organic solvent, the lid moves apart, making the active site accessible. Furthermore, lipases prefer water-insoluble substrates, typically triglycerides composed of long-chain fatty acids, whereas esterases preferentially hydrolyze ’simple’ esters (e.g. ethyl acetate) and usually only triglycerides bearing fatty acids shorter than C6 (Table 1). Both enzymes have been shown to be stable and active in organic solvents, but this feature is more pronounced with lipases. In a recent publication [10], a comparison of amino acid sequences and 3D-structures of lipases and esterases suggested that they can be distinguished by a pH-dependent electrostatic ‘signature’; the active site of lipases displays a negative potential in the pH-range associated with their maximum activity (typically at pH 8), whereas esterases show a similar pattern, but at pH values around 6, which correlates with their usually lower pH-activity optimum [10].

Screening of esterases is usually performed either by employing chromophoric substances (e.g. $p$-nitrophenyl esters) or by using tributyrin-supplemented agar plates. Organisms producing active esterases are then identified by halo formation on plates containing dispersed water-insoluble substrates such as triglycerides. Hydrolysis of these dispersed lipid droplets leads to a clearing zone around the colonies. Then, lipases can be distinguished from carboxyl esterases by their substrate spectra, using $p$-nitrophenyl palmitate (cleaved by lipases) vs. $p$-nitrophenyl butyrate (cleaved by esterases and sometimes also by lipases).

In contrast to this traditional approach, modern molecular biology techniques allow for DNA library expression

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**Table 1**

<table>
<thead>
<tr>
<th>Property</th>
<th>Lipase</th>
<th>Esterase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preferred substrates</td>
<td>Triglycerides (long-chain), secondary alcohols</td>
<td>Simple esters, triglycerides (short-chain)</td>
</tr>
<tr>
<td>Interfacial activation/ld</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Substrate hydrophobicity</td>
<td>High</td>
<td>High to low</td>
</tr>
<tr>
<td>Enantioselectivity</td>
<td>Usually high</td>
<td>High to low to zero</td>
</tr>
<tr>
<td>Solvent stability</td>
<td>High</td>
<td>High to low</td>
</tr>
</tbody>
</table>

---

**Fig. 1.** Schematic presentation of the $\alpha/\beta$-hydrolase fold. $\beta$-Sheets (1–8) are shown as blue arrows, $\alpha$-helices (A–F) as red columns. The relative positions of the amino acids of the catalytic triad are indicated as orange circles.
screening, which also enables access to enzymes of ‘non-culturable’ organisms. By this strategy, for instance, the company Diversa (San Diego, CA, USA) identified 120 unique esterases/lipases from only 16% of the total DNA obtained from an alkaline soil sample [11]. Moreover, many of these enzymes are highly thermophilic, show broad substrate specificity and they belong to 21 protein families.

2. Classification

Classification of enzymes can be done either based on their substrate specificity or by sequence alignments. The former requires that all enzymes which need to be compared have been assayed with the same or at least related substrates, preferentially under similar reaction conditions. Although this would allow the (bio-)chemist to directly identify a suitable enzyme for a given synthetic problem, this is not a common strategy. Due to the increasing availability of sequence information in public databases, the comparison of amino acid sequences can provide a clearer picture about the evolutionary relationship between enzymes of different origin. On the other hand, it often turns out that high sequence homology cannot be related to the enzymes properties (substrate specificity, stereoselectivity, pH and temperature optima, solvent stability), and in some cases completely different types of reactions are catalyzed. For instance, a bromoperoxidase from Streptomyces aureofaciens shares ~55% sequence identity to an esterase from Pseudomonas fluorescens [12,13] but they share little substrate specificity.

Until a few years ago, it was believed that for all lipases and carboxyl esterases only the consensus sequence motif Gly-x-Ser-x-Gly (where x represents an arbitrary amino acid residue) occurs around the active site serine. Indeed, most lipases and esterases contain this motif (Table 2). More recently, a thorough comparison of 53 amino acid sequences of lipases and esterases revealed that other motifs also exist [14]. These have been discussed in detail by Arpigny and Jaeger [14] and only the most important findings will be reviewed here. For instance, some lipases and an esterase from Streptomyces scabies contain a GDSL (Gly-Asp-Ser-Leu) consensus sequence. Moreover, structure elucidation of this esterase revealed that it contains a catalytic Ser-His dyad instead of the common Ser-Asp-His triad [8]. The acidic side chain, which usually stabilizes the positive charge of the active site histidine residue, is replaced by the backbone carbonyl of Trp135 located three positions upstream of the His itself. The enzyme also has an α/β-tertiary fold, which differs substantially from the α/β-hydrolase fold. Other esterases in the GDSL group includes those from Pseudomonas aeruginosa (accession code: AF005091), Salmonella typhimurium (AF047014) and Photorhabdus luminescens (X66379), the first two being outer-membrane-bound esterases. Other enzymes show high homology to the mammalian hormone-sensitive lipase family. Here, conserved sequence blocks were found, which initially have been related to activity at low temperature. However, it was found that esterases from psychrophilic (e.g. Moraxella sp., X53869; Psychrobacter immobilis, X67712) as well as mesophilic (Escherichia coli, AE000153) and thermophilic (Archeoglobus fulgidus, AAE000985) origins belong to this family.

Members of family V, such as esterases from Sulfolobus acidocaldarius (AF071233) and Acetobacter pasteurianus (AB013096), share significant homology to non-lipolytic enzymes, e.g. epoxide hydrolase, dehalogenase and haloperoxidase. Rather small (23–26 kDa) enzymes are found in family VI, which includes an esterase from P. fluorescens, of which the structure is known [15]. The esterase is active as a dimer, has a typical Ser-Asp-His catalytic triad and hydrolyzes small substrates, but not long-chain triglycerides. Interestingly, ~40% homology to eukaryotic lysophospholipases is found for members of this family.

In contrast, esterases from family VII are rather large (~55 kDa) and share significant homology to eukaryotic acetylcholine esterases and intestine or liver carboxyl esterases (e.g. pig liver esterase). A p-nitrobenzyl esterase from Bacillus subtilis [16,17] and an esterase from Arthrobacter oxydans (Q01470) active against phenylcarbamate herbicides [18] belong to this group. In the last family, VIII, high homology to class C β-lactamases is observed. These enzymes contain a Gly-x-Ser-x-Gly motif and a Ser-x-Lys motif, but it has recently been demonstrated by site-directed mutagenesis studies of an esterase (EstB) from Burkholderia gladioli that the Gly-x-Ser-x-Gly motif does not play a significant role in enzyme function [9]. The most prominent member is an esterase from Arthrobacter globiformis (AAA99492) [19], which stereoselectively forms an important precursor of pyrethrin insecticides (see Section 3.1).

More information about the biochemical properties and preferred substrates of selected carboxyl esterases is summarized in Table 2.

3. Application in biocatalysis

3.1. Synthesis of optically pure compounds

Although a considerable number of microbial carboxyl esterases is known which have been overexpressed in suitable hosts (Table 2), only a few of them have been used for the synthesis of optically pure compounds. The major reasons for this are their limited commercial availability and their frequently observed moderate enantioselectivity. Several esterases have been available in recent years from various suppliers (e.g. Fluka, Amano, Jülich Fine Chemicals, Diversa, Roche Diagnostics, Thermogen).

Probably the best studied enzyme is the so-called carboxyl esterase NP (NP from naproxen, a non-steroidal
Table 2
Comparison of various (recombinant) microbial carboxyl esterases

<table>
<thead>
<tr>
<th>Origin</th>
<th>Biochemical properties</th>
<th>Specific substratesb</th>
<th>Remarks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. gladioli ATCC10248 (EstB)</td>
<td>392 aa, 42 kDa</td>
<td>pNP-esters, triglycerides (up to C6), deacylates, cephalosporins</td>
<td>S-x-x-K motif, β-lactamase-like</td>
<td>[9]</td>
</tr>
<tr>
<td>B. gladioli ATCC10248 (EstC)</td>
<td>298 aa, 32 kDa</td>
<td>pNP-esters (up to C5), not triglycerides</td>
<td>G-x-S-x-G motif, homology to plant hydroxynitrile lyases</td>
<td>[65]</td>
</tr>
<tr>
<td>P. fluorescens DSM50106</td>
<td>36 kDa, T&lt;sub&gt;opt&lt;/sub&gt; 43°C</td>
<td>Lactonates, ethyl caprylate, moderate enantioselectivity</td>
<td>G-x-S-x-G motif, homology to a haloperoxidase</td>
<td>[37]</td>
</tr>
<tr>
<td>P. fluorescens SIKW1</td>
<td>27 kDa, homodimer</td>
<td>pNP-esters, high enantioselectivity for α-phenyl ethanol, moderate E values for other alcohols and carboxylic acids</td>
<td>Low haloperoxidase activity, altered substrate specificity and improved enantioselectivity by directed evolution</td>
<td>[12,49,50,53,60,61,66]</td>
</tr>
<tr>
<td>Pseudomonas putida MR2068</td>
<td>29 kDa, homodimer, T&lt;sub&gt;opt&lt;/sub&gt; 70°C</td>
<td>Alkyl-dicarboxylic acid methyl esters, high stereoselectivity (E&gt;100)</td>
<td>–</td>
<td>[58,67]</td>
</tr>
<tr>
<td>Bacillus acidocaldarius</td>
<td>34 kDa, T&lt;sub&gt;opt&lt;/sub&gt; 70°C</td>
<td>pNP-esters (best: hexanoate), moderate stereoselectivity (best: E~18)</td>
<td>Homology to hormone-sensitive lipase</td>
<td>[68]</td>
</tr>
<tr>
<td>B. subtilis NRRL B8079</td>
<td>489 aa, 54 kDa, T&lt;sub&gt;opt&lt;/sub&gt; 52°C (66.5°C for best mutant)</td>
<td>p-nitrobenzyl ester of Loracarbef</td>
<td>Evolved by directed evolution for increased stability in DMF and thermostability</td>
<td>[16,17,38,55]</td>
</tr>
<tr>
<td>Bacillus steareothermophilus</td>
<td>–</td>
<td>pNP-esters, moderate enantioselectivity</td>
<td>Thermostable mutants</td>
<td>[69,70]</td>
</tr>
<tr>
<td>B. subtilis (ThaiI-8) c</td>
<td>32 kDa, T&lt;sub&gt;opt&lt;/sub&gt; 35-55°C</td>
<td>High enantioselectivity towards 2-arylpropionic acids</td>
<td>Structure known, more stable mutants by SDMb</td>
<td>[20]</td>
</tr>
<tr>
<td>Thermosamballibacterium sp. JWSL YS485</td>
<td>320 aa, 36 kDa</td>
<td>Xylose tetra acetate, cephalosporin C, MU-Aceb</td>
<td>G-x-S-x-G motif</td>
<td>[35]</td>
</tr>
<tr>
<td>Actinobacter sp. ADP1</td>
<td>37 kDa</td>
<td>pNP-esters (best: hexanoate), benzyl esters</td>
<td>G-x-S-x-G motif, involved in catechol branch of β-ketoadipate pathway</td>
<td>[36]</td>
</tr>
<tr>
<td>Clostridium thermocellum</td>
<td>31 kDa</td>
<td>Feruloyl esters</td>
<td>Esterase activity within cellulosome</td>
<td>[33]</td>
</tr>
<tr>
<td>Pyrococcus furiosus DSM3638</td>
<td>T&lt;sub&gt;opt&lt;/sub&gt; 100°C, t1/2 50 min at 126°C</td>
<td>MU-Aceb</td>
<td>–</td>
<td>[71]</td>
</tr>
<tr>
<td>Lactococcus lactis c</td>
<td>258 aa, 30 kDa</td>
<td>pNP-esters (best: hexanoate), tributyrin, C&lt;sub&gt;4&lt;/sub&gt;-phospholipids</td>
<td>G-x-S-x-G motif, function unclear</td>
<td>[72]</td>
</tr>
<tr>
<td>Rhodococcus ruber DSM 4338</td>
<td>34 kDa, tetramer</td>
<td>Heroin</td>
<td>Two esterases with opposite enantiopreference</td>
<td>[77]</td>
</tr>
<tr>
<td>Rhodococcus sp. H1</td>
<td>574 aa, 65 kDa, monomer</td>
<td>Cocaine</td>
<td>Two esterases with opposite enantiopreference</td>
<td>[59]</td>
</tr>
<tr>
<td>Streptomyces chrysosporium</td>
<td>42 kDa</td>
<td>pNP-esters (best: butyrate)</td>
<td>G-x-S-x-G motif, high homology to esterase from A. globiformis</td>
<td>[75]</td>
</tr>
<tr>
<td>Streptomyces diastatochromogenes</td>
<td>326 aa, 31 kDa</td>
<td>pNP-esters, moderate enantioselectivity</td>
<td>–</td>
<td>[76,77]</td>
</tr>
<tr>
<td>Oryzomyces sp. PC-2</td>
<td>313 aa, 35 kDa, T&lt;sub&gt;opt&lt;/sub&gt; 30°C</td>
<td>Xylose tetra acetate</td>
<td>G-x-S-y-G motif, homology to X-prolyl-dipeptidyl aminopeptidases</td>
<td>[74]</td>
</tr>
<tr>
<td>Aspergillus awamori IFO4033</td>
<td>275 aa, 31 kDa</td>
<td>Wheat bran, α-naphthol acetate</td>
<td>G-x-S-y-G motif, conserved His&lt;sub&gt;656&lt;/sub&gt;</td>
<td>[73]</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>258 aa, 30 kDa, homodimer, T&lt;sub&gt;opt&lt;/sub&gt; 25°C</td>
<td>Isoamyl acetate, isobutyl acetate</td>
<td>–</td>
<td>[79]</td>
</tr>
</tbody>
</table>

aOverexpressed in E. coli, if not stated otherwise
bPNP, p-nitrophenyl; MU-Ace, 4-methylumbelliferyl-acetate; SDM, site-directed mutagenesis
cNon-recombinant purified enzymes
dOverexpressed in L. lactis
eOverexpressed in B. subtilis

---

[References]
[9, 65, 37, 12, 49, 50, 53, 60, 61, 66, 16, 17, 38, 55, 69, 70, 20, 35, 36, 33, 71, 72, 77, 75, 76, 77, 34, 78, 79]
Carboxylic acids

MeO

O

COOH

Me

R

Me

R

O

CO2H

Carboxyl esterase NP, (S)-Naproxen, high E [20]

Carboxyl esterase NP, high E [21,22]

Carboxyl esterase NP, high E [57]

MeO(CH2)2

COOH

PPE, high E, n=0 or 1 [58]

COOH

PFE-mutant, E=12, [53]

PFE-mutant, E=90, [53], Kaunas and Bornscheuer (unpublished)

Primary alcohols

HO

PAE, E=12, [62]

BCE, E>100 [63]

Secondary alcohols

HO

BGE, E>100, [64]

PFE, E>100, [49,50]

Tertiary alcohols

HO

RRE, (S)- or (R)-Inabiol, high E [59]

HO

Esterases can be also employed in reactions where chemo- or regioselectivity is of interest. The most prominent example is the use of carboxyl esterases in the release of ferulic acid from plant cell wall polysaccharides such as pectin or xylan. In xylans, ferulic acid is attached to arabinoxylans, and the enzyme was cloned in E. coli [24]. In a 160-g scale process, hydrolysis was performed at pH 9.5 at 50°C. Acid produced was separated through a hollow-fiber membrane module and the esterase was very stable over four cycles of 48 h [19].

Further selected examples for the application of microbial carboxyl esterases in the synthesis of optically pure compounds are summarized in Fig. 2.
binose residues, which are bound to the xylan backbone. In pectins, ferulic acid can be linked to galactose or arabinose in side chains. Ferulic acid thus obtained can be converted enzymatically into vanillin, a major flavor compound [25–27]. Feruloyl esterases have been isolated from a wide range of microorganisms [28–33]. Some of these esterases have been overexpressed in heterologous hosts and their properties are summarized in Table 2. In addition, the 2-O or 3-O positions of arabinose are often acetylated and the acetyl group can be also removed by the action of specific acetyl xylan esterases [34,35]. Properties of some of these recombinant enzymes are given in Table 2.

Microbial carboxyl esterases are also involved in the catabolism of aryl esters as shown for an enzyme found in Acinetobacter sp. [36], which releases an acid from benzyl esters. The resulting benzyl alcohols are then oxidized to benzoates by dehydrogenases and further metabolized in the β-ketoacid pathway. For a lactone-specific esterase from P. fluorescens, it was proposed that the enzyme is involved in the hydrolysis of lactones formed by Baeyer–Villiger monooxygenases [37]. In the case of cyclohexanone, the resulting α-hydroxy acids can then be further metabolized to adipate.

An application of considerable industrial interest is the mild removal of protecting groups as shown for a p-nitrobenzyl esterase from B. subtilis, which specifically removes this residue from the antibiotic Loracarbef [16]. As the enzyme is only weakly stable in dimethyl formamide, the esterase was improved by directed evolution (see Section 4.2).

4. Directed evolution of microbial esterases

4.1. Concept of directed evolution

Directed evolution (also called evolutive biotechnology or molecular evolution) is a technology developed in the early 1990s to generate desired enzyme variants or, more recently, to evolve metabolic pathways. Usually, random mutagenesis of the gene encoding the protein is performed by error-prone PCR, leading to huge libraries of mutants. Other methods are based on random recombination of DNA fragments such as DNA (or gene) shuffling, the Staggered Extension Process or random primer recombination. Libraries thus created are then assayed by high-throughput techniques to identify improved variants, as the huge number of mutants cannot be analyzed by common analytical tools such as gas chromatography and high-performance liquid chromatography. These are too time-consuming and expensive and therefore spectrophotometric methods are frequently used, especially photometric and fluorimetric assays performed in microtiter plate-based formats in combination with high-throughput robot assistance.

Prerequisites for directed evolution are the availability of the gene(s) encoding the enzyme(s) of interest, a suitable (usually microbial) expression system, an effective method to create mutant libraries which are then assayed by a suitable screening or selection system. In contrast to rational protein design, the knowledge of the 3D structure of the enzyme and its mechanism of catalysis are not necessary. It should also be emphasized that a mutant library, once created, can be stored and subjected to new screening rounds if a new problem to be solved comes up.

Detailed overviews about methods for directed evolution as well as various examples for its successful application can be found in a considerable number of recent reviews [11,38–48].

4.2. Alteration of substrate specificity and enantioselectivity

Due to its very recent development, only a few examples for the directed evolution of esterases can be found. In my own group, we succeeded in altering the substrate specificity of an esterase from P. fluorescens (PFE) [49,50] by these methods. A sterically hindered 3-hydroxy ester not accepted as substrate by 20 wild-type hydrolas was stereoselectively hydrolyzed by a double-mutant of PFE generated using the mutator strain Epicurian coli XL1-Red [51,52]. The key to the identification of improved variants was an agar plate-assay system based on pH indicators, thus leading to a change in color upon hydrolysis of the ethylester. Parallel assay of replica-plated colonies on agar plates supplemented with the glycerol derivative of the 3-hydroxy ester was used to refine the identification, because only E. coli colonies producing active esterases had access to the carbon source glycerol, thus leading to enhanced growth and in turn larger colonies. Although this method was useful to turn an inactive wild-type enzyme into an active esterase, the best mutant showed only moderate enantioselectivity (E~5).

Thus, in the next step, enantioselectivity of PFE was improved by directed evolution. Mutant libraries were created by error-prone PCR and by using the mutator strain. An extremely accurate determination of the enantioselectivity was achieved by using resorufin esters of (R)- or (S)-3-phenylbutyric acid, which allowed measurement of fluorescence in microtiter plates avoiding problems with interfering compounds present in the culture medium [53]. This led to the identification of variants, which exhibited enantioselectivities of E=5.2–6.6 after a single round of mutation compared to an E of 3.5 for the wild-type enzyme. A further increase in enantioselectivity up to E=12 was achieved by saturation mutagenesis at all three positions identified for the first-generation mutants (E. Henke and U.T. Bornscheuer, unpublished). However, combinations of the best variants by site-directed mutagenesis gave no further improvement (Figs. 2 and 3). It should be noted, that apparent E values (E_app) determined in microtiter plates were quite close to E_true values, which have been
determined by gas chromatographic analysis of samples from resolutions of racemic mixtures of the corresponding ethylesters of 3-phenylbutyric acids. Here, competition between the two enantiomers is also included, whereas the microtiter plate-assay delivers only apparent $E_{\text{app}}$ values.

Significantly higher increases in enantioselectivity were achieved for the resolution of methyl 3-bromo-2-methylpropionate (Fig. 2). Using a homology model of PFE, Trp29 and Phe199 were identified as positions for random mutagenesis. Libraries were then screened using Quick E [54], which is based on hydrolysis of optically pure (R)- or (S)-resorcin ester [53]; GC: $E$ values ($E_{\text{true}}$) calculated according to literature [56] from data determined by gas chromatographic analysis on a chiral column from samples obtained after esterase-catalyzed hydrolysis of (R,S)-3-phenyl butyric acid ethylester [50].

5. Concluding remarks

It was shown in this review, that a significant number of microbial carboxyl esterases has been discovered so far and many of them have been overexpressed, enabling, in principle, production on a commercial scale. Moreover, our knowledge of their evolutionary relationships and the recent discovery of different consensus sequence motifs added considerably to our understanding of their functions. The large diversity of reactions and substrates handled by esterases in nature is still poorly explored. In part, this is caused by their limited commercial availability and the extensive use of many lipases in biocatalysis. These ‘competing’ existing enzymes often show higher enantioselectivity, stability in organic solvents and a broader substrate specificity than the esterases. However, modern methods of enzyme engineering – especially directed evolution – will certainly provide suitable carboxyl esterase variants with increased use in organic synthesis and other areas of application in the near future. The few examples of successfully evolved carboxyl esterases already show that this goal is indeed achievable.

Acknowledgements

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Clostridium thermocellum
cellulosome can be attributed to previously unknown domains of

XynY and


erization of an acetyl xylan esterase from the anaerobic fungus


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