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# Biotechnological Application of Cutinase: A Powerful Tool in Synthetic Biology

**Xiuhong Liang and Huibin Zou ***

State Key Laboratory Base of Eco-Chemical Engineering, College of Chemical Engineering, Qingdao University of Science and Technology, Qingdao 266043, China

* Correspondence: zouhb@qibebt.ac.cn or huibinzou@qust.edu.cn

**Abstract:** Cutinases (EC 3.1.1.74) are widely distributed in fungi, bacteria and plants with diversified structures and properties. Besides acting on the natural substrate cutin, cutinases are the first line of natural biocatalysts to hydrolyze artificial polyesters and toxic xenobiotics such as polyethylene terephthalate (PET), polycaprolactone (PCL), polylactic acid (PLA), polyhydroxybutyl succinate (PBS), phthalate and malathion esters. Moreover, cutinases can act as promising stereoselective catalysts in esterification and transesterification reactions and present better selectivities than lipases. These pioneering studies indicate that the biotechnological application of cutinase as a powerful tool in synthetic biology deserves further investigation, for both degradation and biosynthesis towards a broader range of ester bond-containing substrates. This review summarizes the classifications and properties of cutinases from different sources and insights into the structure–function relationship of different cutinases. It also highlights the uniqueness and advantages of representative cutinases in biodegradation and biosynthesis, and then prospects the future application of natural and engineered cutinases in synthetic biology.

**Keywords:** cutinase; biodegradation; biosynthesis



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## 1. Introduction

Cutinase is detected by its ability to degrade cutin, which is an aliphatic polyester and acts as the natural protection barrier of many plants [1]. Natural cutinases are mainly related to the pathogenicity of plant pathogens, which can break the cutin barrier to provide carbon sources [2,3]. Cutinases are also found in plants, which can degrade the cuticle barrier of stigma to promote the fertilization process [4].

As the substrate of cutin is an insoluble lipid polyester [5], cutinase is classified as a member of the α/β hydrolase family together with lipase and esterase [6]. Compared to other hydrolase family members, cutinase has some specific characteristics. (1) Cutinase has the smallest molecular weight among the α/β hydrolase family members [6]. (2) Although both cutinase and lipase have the Ser-His-Asp catalytic triad, cutinase does not have the hydrophobic lid covering the active site serine (the lid is present in lipase); thus a broad range of substrates other than cutin can be recognized by cutinase [5].

It is speculated that cutinases have undergone diversified evolution and thus possess variable functions [7]. For example, the biodegradation ability of cutinase cannot only be applied to degrade large molecular weight cutin and synthetic plastics [8] but can also be applied to degrade low molecular weight esters, and short-chain and long-chain triacylglycerols [5]. In addition, cutinases can be applied as functional biosynthetic tools in esterification and transesterification [9,10]. This review summarizes the natural resources and characteristics of variable cutinases, pointing out their key characteristics and reviewing their updated application in biodegradation and biosynthesis. Moreover, the review also speculates on future trends in engineering cutinases for broader biotechnological applications.

## 2. Diversified Cutinases from Different Sources

Earlier studies have identified a broad range of cutinases from fungi, bacteria and plants [6,11,12], which can target variable substrates such as cutin, plant oils or unnatural polyesters [13]. Fungi and bacterial cutinases have attracted more interest (as summarized in Table 1), and bioengineering efforts have been applied to gain engineered cutinases with increased catalytic efficiencies and stabilities which can meet the requirements of industrial applications [14]. The fungi, bacteria and plant cutinases present different characteristics, as summarized in Table 2.

**Table 1.** Cutinases from fungal and bacterial sources.

| Source | Organism | NCBI No. | Substrate |
|---|---|---|---|
| Fungi | *Alternaria brassicicola* cutinase | AAA03470.1 | Cutin |
| | *Aspergillus nidulans* cutinase | ABF50887.1 | PET |
| | *Aspergillus niger* cutinase | CAL00335.1 | PET |
| | *Aspergillus niger* cutinase 2 | AKE48475.1 | PET |
| | *Aspergillus niger* cutinase 3 | AKA62190.1 | PET |
| | *Aspergillus oryzae* cutinase | BAA07428.1 | Cutin |
| | *Aspergillus oryzae* cutinase 2 | ALB07219.1 | Cutin |
| | *Colletotrichum capsici* cutinase | ADQ27862.1 | Cutin |
| | *Colletotrichum fiorinae* cutinase | EXF73863.1 | Cutin |
| | *Colletotrichum fructicola* cutinase | ELA29687.1 | Cutin |
| | *Colletotrichum gloeosporioides* cutinase | AAL38030.1 | Cutin |
| | *Colletotrichum incanum* cutinase | KZL82629.1 | Cutin |
| | *Colletotrichum orbiculare* cutinase | TDZ15371.1 | Cutin |
| | *Colletotrichum orchidophilium* cutinase | XP_022472246.1 | Cutin |
| | *Colletotrichum salicis* cutinase | KXH52034.1 | Cutin |
| | *Colletotrichum sidae* cutinase | TEA20600.1 | Cutin |
| | *Colletotrichum simmondsii* cutinase | KXH53950.1 | Cutin |
| | *Colletotrichum spinosum* cutinase | TDZ13928.1 | Cutin |
| | *Colletotrichum trifolii* cutinase | TDZ54558.1 | Cutin |
| | *Colletotrichum truncatum* cutinase | P10951.1 | Cutin |
| | *Cryptococcus* sp. cutinase | BAK82405.1 | Cutin |
| | *Fusarium petroliphilum* cutinase | AAB05922.1 | Cutin |
| | *Fusarium solani pisi* cutinase | AAA33334.1 | PCL |
| | *Fusarium solani pisi* cutinase 2 | AAL18696.1 | PCL |
| | *Humicola insolens* cutinase | QAY29138.1 | PET |
| | *Magnaporthe grisea* cutinase | EHA46959.1 | Cutin |
| | *Monilinia fructicola* cutinase | AAZ95012.1 | Cutin |
| | *Penicillium* sp. 2HH cutinase | KAF7739429.1 | Cutin |
| | *Pseudozyma antarctica* cutinase | GAC73680.1 | Cutin |
| | *Pyrenopeziza brassicae* cutinase | CAB40372.1 | Cutin |
| | *Thermothielavioides terrestris* cutinase | ATP16782.1 | Cutin |
| Bacteria | *Botrytis cinerea* cutinase | CAA93255.1 | Cutin |
| | *Ideonella sakaiensis* PETase | GAP38373.1 | PET |
| | Leaf and branch compost cutinase (LCC) | AEV21261.1 | PET |
| | *Mycobacterium tuberculosis* cutinase | CEL55977.1 | Cutin |
| | *Thermobifida alba* AHK119 Tha-Cut1 | ADV92525.1 | PET and PLA |
| | *Thermobifida cellulosilytica* Thc-Cut1 | ADV92526.1 | PET |
| | *Thermobifida cellulosilytica* Thc-Cut2 | ADV92527.1 | PET |
| | *Thermobifida fusca* cutinase | AAZ54921.1 | PET |
| | *Thermobifida fusca* TfCut1 | CBY05529.1 | PET |
| | *Thermobifida fusca* TfCut 2 | CBY05530.1 | PET |
| | *Thermobifida fusca* Thf42-Cut1 | ADV92528.1 | PET |
| | *Thermobifida fusca* KW3 TfCa cutinase | CAZ65068.1 | PET |
| | *Thermobifida fusca* YX Tfu-0882 cutinase | AAZ54920.1 | PET |
| | *Thermomonospora curvata* Tcur0390 cutinase | ACY95991.1 | Cutin |
| | *Thermomonospora curvata* Tcur1278 cutinase | ACY96861.1 | Cutin |

**Table 2.** Characteristics of cutinases from different sources.

| Source | Size | Optimum Temp | Optimum pH | Inhibitors |
|---|---|---|---|---|
| Fungi | 22–26 kDa | 30–40 °C | 10.0 | Serine-directed reagents |
| Bacteria | 30 kDa | 40–60 °C | 8.5–10.5 | Serine-directed reagents |
| Plant | 40 kDa | 30–40 °C | 6.8–8.0 | Thiol-directed reagents |

### *2.1. Fungal Cutinases*

Filamentous fungi have the largest cutinase library [15]. The molecular weight of fungal cutinase tends to be 22–26 kDa [16], which is less than those of bacteria and plant cutinases. However, experimental determination found a difference between theoretical and experimental Mw. The reason for the deviation between the experimental results and the theoretical prediction may be due to the post-translational modification of cutinases. It is suspected that the N-terminus of cutinase may be blocked by glucuronidation [17]. In addition, post-translational phosphorylation modifies the molecular weight; nearly all fungal cutinase contain at least one phosphorylation site [18].

Insights into the structure of fungal cutinases, the conserved sequences of GXSXG (usually GYSQG), the catalytic triangle of S-H-D and at least one phosphorylation site have been found. In addition, fungal cutinases have two pairs of disulfide bonds, four involved cysteines located near the N-terminus and the catalytic triangle S-H-D. Tyrosine, phenylalanine and tryptophan involved in sugar binding are highly conserved at the C-terminus of fungal cutinases [19,20].

As a model enzyme, the cutinase from *Fusarium solani pisi* (*Fsp*) has been extensively studied [12]. Glucuronic acid-based glucuronidation (via an amide bond) was found at the N-terminus of *Fsp* cutinase, and carbohydrates were connected by the o-glycosidic bonds of serine, threonine, β-hydroxyphenylalanine and β-hydroxytyrosine [19]. The detailed structural and catalytic characteristics of *Fsp* cutinase provided useful reference for the rational engineering of wild-type cutinase to further improve the performance of engineered *Fsp* cutinase [12].

There are two pairs of disulfide bonds in the structure of fungal cutinase, which play a significant role in the stability of fungal cutinase. When fungal cutinase takes cutin as the substrate, the optimum pH is 10.0, and the optimum temperature is 30–40 °C [11]. Fungal cutinase can be inhibited by active serine-directed reagents, such as diisopropyl fluorophosphate but not by thiol-directed reagents [6]. Some fungal cutinases (such as cutinase from *Aspergillus nidulans*) can be induced by oil, triacylglycerides and fatty acids while inhibited by glucose and other sugars [17].

### *2.2. Bacterial Cutinases*

In addition to the most studied fungal cutinases, bacteria-derived cutinases are attracting interest due to their unique properties [21]. In terms of catalytic mechanism, the cutinases of bacteria and fungi present high similarity; both of which have the active serine catalytic triad [22]. However, bacterial cutinases present a different amino acid composition than fungal and plant cutinases, especially in the composition of methionine, histidine and lysine residues. For example, only one or two histidine residues are found in fungal cutinases, while bacterial cutinases can have up to five histidine residues [23]. Due to the difference of amino acid composition, the optimal pH (between 8.5 and 10.5) for bacterial cutinase is different to that of fungal cutinase. More importantly, it has been found that bacterial cutinases have much better thermal stability than the cutinases derived from fungi [23].

The cutinase from *Pseudomonas putida* (a plant parasitic bacterium) is one of the well-studied bacterial cutinases. The molecular weight of *P. putida* cutinase is around 30 kDa, and it presents higher thermos-stability than fungal cutinases [22]. Another "star" bacterial cutinase, the LC cutinase (isolated from leaf compost) can degrade synthetic polyester under high temperature conditions [24,25]. Obtaining this strain from plant leaves, it was found that this bacterium has a mutually beneficial symbiosis with another micro-organism called nitrogen-fixing *Corynebacterium* sp. During mutualism, *P. putida* provides a carbon source by hydrolyzing the leaf cutin, while nitrogen-fixing *Corynebacterium* sp. is dedicated to providing a source of nitrogen [26].

Bacterial cutinase can be inhibited by active serine-directed reagents (including organophosphates and organoboronic acids). This mechanism can confirm the "active serine" character of bacterial cutinase. However, thiol-directed reagents and metal ion chelators will not adversely affect its activity [6].

### *2.3. Plant Cutinases*

Plant cutinases present different characteristics than those of microbial sources (Table 2). In plant fertilization, as the stigma is surrounded by a continuous cuticle, the pollen tube can only grow downwards after passing through the barrier [4]. Plant cutinase can degrade the cuticle to make the pollen tube pass through the barrier [27]. A pioneering study purified a cutinase from pollen and concluded that its molecular weight (40 kDa) was different to those of fungi and bacterial cutinases [28]. The titratable acidity was increased after adding cutin to the germinated pollen, which further verified the presence of plant cutinase [29].

When cutin was hydrolyzed, the optimum pH of pollen cutinase was 6.8. When p-nitrophenyl esters of fatty acids were applied as the substrate, the optimum pH was 8.0 [6,28]. Plant cutinase can be strongly inhibited by thiol-directed reagents such as N-ethylmaleimide and p-hydroxymercuribenzoate and will not be affected by an active serine-directed reagent, such as diisopropyl fluoro phosphate [6]. Purified plant pollen can catalyze the hydrolysis of primary alcohol esters but presents low activities towards tripalmitoyl and trioleylglycerol [6,28,29].

In terms of amino acid composition, pollen cutinase also presents its uniqueness from microbial cutinase. Compared to the amino acid composition of fungal-derived cutinase, pollen cutinase has relatively higher acidic amino acids and cysteine residues [30]. In addition, the carboxyl content of pollen cutinase is also higher than that of fungal-derived cutinase. Therefore, electrostatic repulsion may affect the degradation of cutin by pollen cutinase but will not affect the degradation of fungal cutinase with a pH value above 7.0 [28].

### *2.4. Comparison of Cutinases from Different Sources*

In summary, fungi, bacterial and plant cutinases present highly diversified structures, which indicate their different catalytic functions and properties. Comparison of the structures of different cutinases can provide insights for the rational engineering of robust cutinases with higher efficiency and stability.

For example, indicated by the disulfide bridge in the structure of fungi *Fsp* cutinase (PDB:2CUT), the bacterial LC cutinase was rationally engineered into ICCG cutinase (D238C and S283C) with a disulfide bridge in its structure (PDB:7W1N) with higher thermal stability [31]. In addition, the cutinase with a neutral charged surface of the "crowning area" near the conserved active sites presents better thermal stability [32]. More methionine, histidine and lysine residues in cutinase can also attribute higher thermal stability, as indicated by the higher thermal tolerance of some bacteria cutinases [23].

Plant cutinases present larger structures than those of bacterial and fungal cutinases (Table 2). The primary sequence of plant cutinase indicates that plant cutinase may belong to the GDSL lipase/esterase family rather than the alpha/beta hydrolase superfamily [33]. However, no plant cutinases have been characterized by their structure information. Indicated by the specific thiol-directed inhibitors, plant cutinases can be classified as "thiol cutinase", and the catalytic center may utilize cysteine rather than serine as the active residue. More studies are needed to further clarify the structure and biochemical property of plant cutinase.

## 3. Applications of Cutinases

### *3.1. Traditional Applications*

As presented in Figure 1, cutinase is one of the popular industrial enzymes in many fields. Industrial cutinases have a broad spectrum of substrates, present high efficiency and stability, and are compatible with other enzymes and reagents.

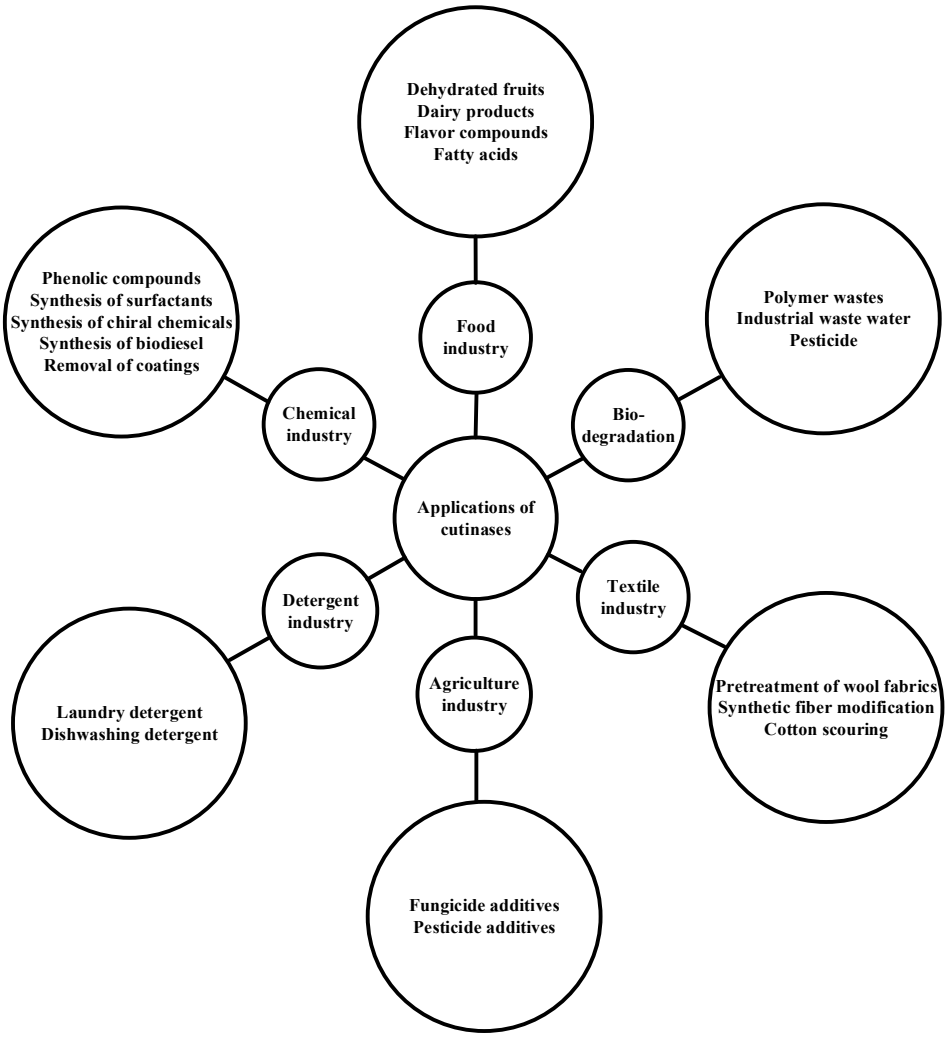


**Figure 1.** Broad applications of cutinase.

Industrial cutinase was first applied in the textile industry [34], in which cutinase can participate in cotton scouring, improving the wettability of cotton fibers [34]. The combination of cutinase and pectinase in cotton fiber scouring can reduce the cost and wastewater treatment by replacing the previously used method of alkali washing [35,36]. In addition, cutinase can be applied in the processing of synthetic fibers and leather: replacing the traditional physical and chemical methods, simplifying the operation steps, reducing environmental pollutions, and reducing energy consumption and costs [34].

Cutinase was also traditionally applied in the food industry. Cutinase presents excellent performance in processing dairy products, flavoring agents and dehydrated fruits [37]. In addition, cutinase can increase the efficiency of pesticides to increase the yields of crops and reduce the pesticide consumption [38]. In sugar factories, cutinases immobilized by chitosan present high efficiency in the decolorization of molasses wastewater, and the decolorization rate can reach 80% [39].

Moreover, cutinase can be applied: as laundry and dishwashing detergents [40]; in the treatment of plant cuticle for the extraction of plant natural ingredients [41]; and in the hydrolysis of papermaking sticky during the papermaking process [42].

A more recent trend in cutinase research has largely expanded its traditional applications. Natural or rationally engineered cutinase can be utilized as a powerful bio-catalytic tool (in biosynthesis or biodegradation) towards unnatural substrates. For example, cutinase can be applied in the synthesis of agrochemicals containing one or more chiral cen-

ters [41] and in the bio-degradation of synthetic contaminations and polymers (detailed information in Sections 3.2 and 3.3).

### *3.2. Ecological Restoration and Biodegradation*

#### 3.2.1. Degradation of Toxicants

Cutinase has been applied in the biodegradation of toxic chemicals or pollutions which have ester structures [43]. For example, *Fsp* cutinase presents an excellent degradation ability towards phthalate esters (phthalates) such as dipentyl phthalate [44], butyl benzyl phthalate [45] and di-(2-ethylhexyl)-phthalate [46]. Although esterases can also degrade phthalate esters, they are less effective than cutinase and can produce more toxic end products [43].

Fungal cutinase also presents higher efficiency than yeast esterase in degrading malathion [47], an organophosphate insecticide and acaricide [48] which has severe toxic effects [49–51]. Another advantage of cutinase is that it produces less toxic end products than esterase in malathion degradation [47].

Other ester-bond containing toxicants can also be hydrolyzed by cutinase. For example, cutinase from thermophilic fungus *Humicola insolens* can degrade 81% zearalenone [52] and 51% aflatoxin [53]. Although the end products were not reported in these studies.

The incomplete hydrolysis of these ester bond-containing pollutants or toxicants by cutinase indicated that further studies are needed to screen more robust cutinase (from bacteria or plant resources) and construct engineered cutinase with improved performance.

#### 3.2.2. Degradation of Polyesters

Of the polyester products consumed, it is estimated that 79% ends up in landfills or is left in the environment [54], resulting in severe environmental issues [55–58]. Thus the biodegradation of polyesters and plastics is one of the most popular directions of recent cutinase studies [57]. As summarized in Figure 2, a broad range of cutinases present degradation abilities towards commercial polyesters such as polyethylene terephthalate (PET), polycaprolactone (PCL) and polylactic acid (PLA), all of which are artificial polymers.

Although PCL is a biodegradable polyester [59], its natural degradation rate is low due to its high hydrophobicity and crystallinity [60]. *Fsp* cutinase can break the hydrophobic barrier and promote the degradation of PCL [61], and the degradation reaction prefers basic environments [62].

More studies focused on the degradation of PET (Figure 2), a durable synthetic polyester which is a popular polyester and is hard to degrade in nature. Although PET wastes can be recycled by the thermomechanical method, the thermal recycled-PET presents decreased mechanical properties, compared to newly synthesized PET [10]. PETase-based treatment of PET wastes is another promising strategy for PET recycling, in which PET wastes are hydrolyzed into monomers for PET polymerization [8,57]. As presented in Figure 2, both fungal and bacterial cutinases present PETase activity. As higher temperatures (around 65) can promote the hydrolysis of PET, thermophilic cutinase is superior to mesophilic cutinases in practical applications [63–65], such as the LC cutinase (isolated from leaf compost) which can maintain activity under high temperatures [24,25]. In order to further improve the hydrolysis efficiency towards PET of natural cutinase, engineered cutinase have been developed. For example, a recent study engineered a robust cutinase which can degrade 90% of PET wastes into monomers within 10 h [8], indicating the advantage of cutinase in the biodegradation of PET wastes.

Cutinases can also degrade PLA, an artificial polyester made from renewable lactic acid [66]. Although PLA is biodegradable, its degradation rate is slow in mild conditions [67]. A recent study characterized a bacterial cutinase (from the compost strain *Thermobifida alba* AHK119) which can effectively degrade PLA [68]. Similar to PLA, polyhydroxybutyl succinate (PBS) is also an artificial and bio-derivable polyester, which is more durable than PLA in mild conditions [69]. Another bacterial cutinase (from *Amycolatopsis mediteranei*) presents biodegradation activity towards PBS and PCL but cannot degrade PLA and PET [70]. The specificity of cutinase can be useful in processing functional polyester

products. For example, *A. mediteranei* cutinase can be applied to process PBS/PLA material to make porous polyester membrane [71].

Another advantage of cutinase in the degradation and recycling of polyester wastes is that cutinase can recognize phthalate esters, thus can remediate the phthalate plasticizers [72] in the process [72].

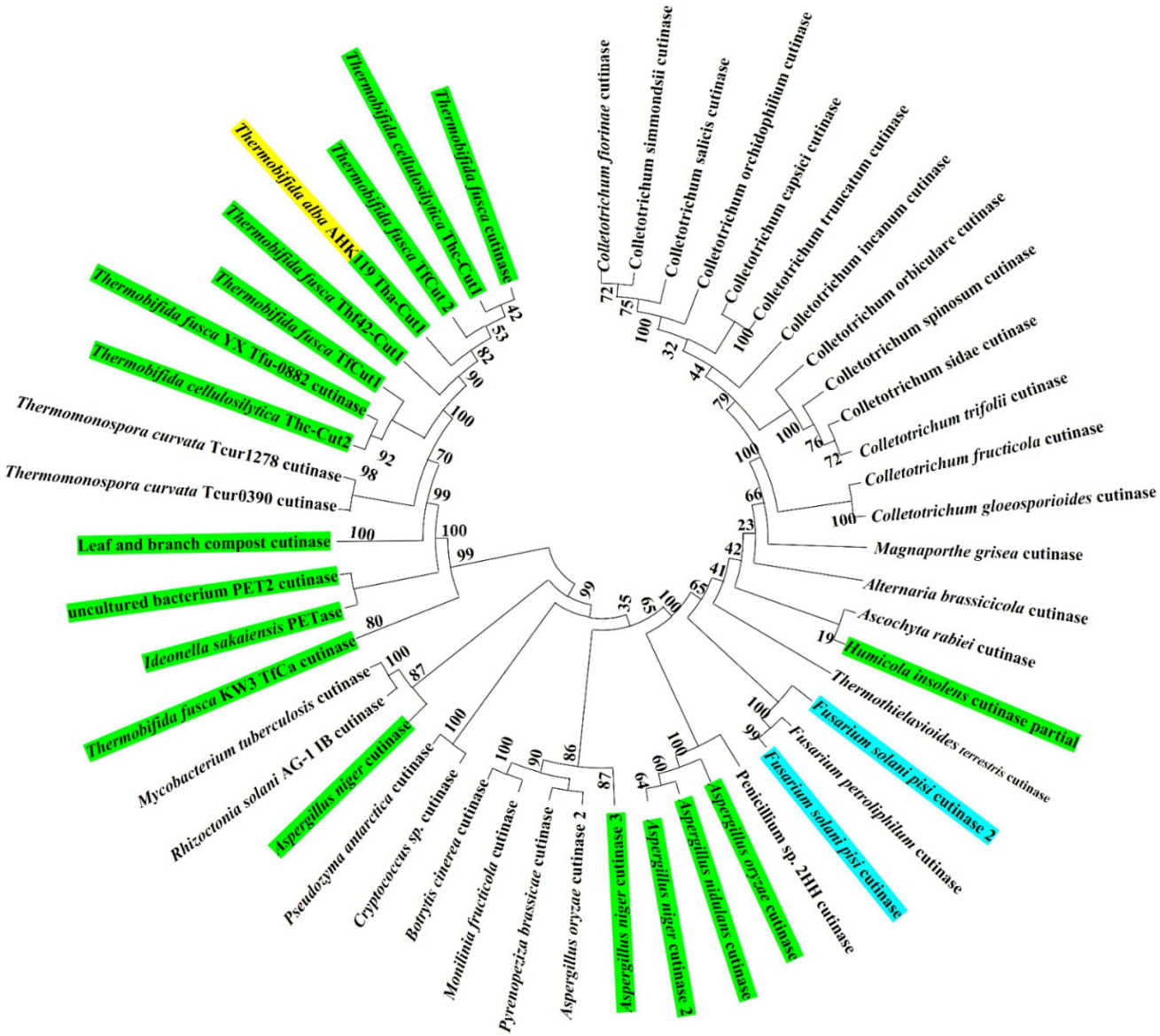


**Figure 2.** Phylogenetic tree of cutinase. The picture is drawn using the software MEGA (version 11, 64 bit), and the sequences were compared by ClustalW. Green background represents cutinases that can degrade PET. Blue background represents cutinases that can degrade PCL. Yellow background represents cutinases that can degrade PLA, and other cutinases without color-label can degrade the natural substrate cutin.

### *3.3. Biosynthesis*

As the reverse reaction of biodegradation, cutinases can also act as biocatalysts in esterification and transesterification reactions to produce polyamides and polyesters [43]. For example, cutinases from *F. solani pisi* and *Burkholderia cepacia* were applied to catalyze the esterification reaction, and the studies showed that the catalytic efficiency of cutinase was affected by low pH (with a higher concentration of substrate) [73,74]. Therefore, the

tolerance of cutinase to acidic environments becomes a major factor in selecting feasible cutinase as esterification catalysts.

To increase the tolerance to acidic substrates and stability in extreme conditions, the immobilization strategy can effectively improve the performance of cutinase during the synthesis reaction for polyester products [75–77]. Among the immobilization methods, entrapment or covalent bonding to various supports were often utilized in immobilized cutinases [43]. The immobilized cutinases presented promising thermal stabilities and continued their activities under a high temperature, and the catalyzed polymerization process under a higher temperature condition can overcome the diffusional limitations in polyester production [43].

Moreover, cutinases can act as stereoselective catalysts in biosynthesis [41,78]. A recent study demonstrated that cutinases from *Aspergillus oryzae* and *Humicola insolens* present better enantioselective activities than lipases in biosynthesis with bulky and sterically hindered substrates [78], indicating that cutinase can perform as a powerful tool in the stereoselective synthesis of pharmaceuticals and other fine chemicals containing ester bonds.

## 4. Frontier Methods in Engineering Robust Cutinases

Enzyme engineering methods were often applied to further improve the efficiency of cutinase in variable cases. For example, cutinase was fused with a binding module to promote the hydrolysis towards PET [79]. To further improve the thermal stability, engineering additional disulfide bonds in proper residues of cutinase can improve catalytic efficiency under extreme conditions [80]. Cutinase was also engineered in *Bacillus subtilis*, which can effectively improve the hydrolysis efficiency of chassis strain in the microbial degradation of polyester wastes [81].

The recent development of silico facilitated methods has prompted the rational engineering and construction of robust cutinases, which have significantly saved time and efforts compared to traditional engineering methods. For example, deep learning-based prediction and experimental testing of positive mutations have been applied in engineering robust cutinases and other biocatalysts with improved catalytic efficiency (degradation of PET) and stability under a wide range of temperatures and pH values [82]. Understanding the structure–functional relationship of variable cutinase [83] will provide more self-learning data for algorithms for further rounds of the design/test cycle. These frontier methods may not only be applied in engineered current cutinases but also in the construction of artificial cutinases with novel functions.

## 5. Summary and Future Prospect

Similar to lipases, cutinases have highly diversified sources (fungi, bacteria and plant), properties and functions. The widely distributed cutinases can catalyze both the ester bond cleavage and formation towards a variety of substrates. In some cases, cutinases have advantages compared to lipases, such as in the biodegradation of durable PET polymers with tough structures, and in the stereoselective synthesis of ester-containing chemicals, cutinases present a better performance than lipases [31,78,82]. These pioneering studies indicated that cutinase has great potential to be applied as a powerful candidate tool in the broader area of synthetic biology.

This review only summarized limited cases in applying cutinases as functional biocatalysts. Studies to screen more robust cutinases or to apply engineering approaches and efforts to improve the catalytic performance of current cutinases are on the rise. Achieving more biodegradation and biosynthesis goals through frontier methods (deep learning- based silico design and experimental testing) is a trend in the research of functional cutinases.

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