Research Progress on bacterial cutinases for plastic pollution

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Abstract. Cutinases (3.1.1.74) are serine esterases that belong to the α/β hydrolase family. Such enzymes are usually produced by phytopathogenic microorganisms in order to penetrate their hosts. Cutinase can degrade the stratum corneum in the leaves or the keratin of the cork in the bark. Cutinase hydrolyzes soluble esters, insoluble triglycerides and various polyesters. In addition to the hydrolysis reaction, cutinase also shows synthetic activity and transester activity. Therefore, as a multifunctional enzyme, cutinase has many fields of application. In recent years, it has been found that cutinase can biodegrade plastic and biomodifie synthetic fibers. Cutinase is the most important enzyme in solving plastic pollution.

1. Introduction
Cutinase received attention first because of its phytopathogenicity, which belongs to serine esterase and has an α/β folding structure [1]. Cutinase can degrade the stratum corneum in the leaves or the keratin of the cork in the bark. It produces a large amount of fatty acid monomers when hydrolyzing the ester bond of the keratin, and can also catalyze the esterification of fatty acids and alcohols. In recent years, the study of bacterial cutinase has become more and more extensive. At present, the bacterial cutinase reported in the literature mainly comes from the genus Thermobifida [2], Pseudomonas [3] and Streptomyces [4].

A large number of bacterial cutinase genes have been cloned and sequenced, and these amino acid sequences have high homology. T. fusca cutinase is the most studied strain of actinomycetes. Chen et al. cloned Tfu_882 and Tfu_883 two cutinase enzymes from the T. fusca strain and found that the amino acid sequence of Tfu_883 is identical to the hydrolase BTA-1. Hu et al. cloned a new cutinase-Est119 from T. alba AHK119, which has two tandem cutinase genes est119 (EST2) and EST1, respectively. On the other hand, Herrero Acero et al. cloned two bacterial cutinase enzymes, Thc_Cut1 and Thc_Cut2, from T. cellulosilytica DSM44535, the amino acid sequences of which are identical to the amino acid sequences of Tfu_0883 and Tfu_0882, respectively. Tcur1278 and Tcur0390 are two cutinase enzymes derived from Thermomonospora curvata DSM 43183, both of which have 62% sequence identity to BTA-1 [12, 13]. Table 1 summarizes the reported bacterial cutinase.

2. Source of cutinase
Cutinase belongs to serine esterase and has an α/β folding structure, which has received attention first because of its phytopathogenicity. According to the source classification of cutinase, cutinase can be divided into bacterial cutinase, fungal cutinase and pollen cutinase. In recent years, the study of bacterial cutinase has become more and more extensive [5-8]. At present, the bacterial cutinase reported in the literature mainly comes from the genus Thermobifida [2], Pseudomonas [3] and Streptomyces [4].

Table 1 summarizes the reported bacterial cutinase.
Table 1. Bacterial cutinase.

| Enzyme      | Source                          | Year  | Uniport Accession number | MW (kDa) | References |
|-------------|---------------------------------|-------|--------------------------|----------|------------|
| PETase      | (Ideonella sakaiensis (strain 201-F6)) | 2016  | GAP38373.1                | 28.6     | [5]        |
| Est119      | Thermobifida alba/AHK119         | 2012  | BAI99230.2               | 26       | [10]       |
| Tha_Cut1    | Thermobifida alba                | 2011  | ADV92525.1               | 28.1     | [14]       |
| Thh_Est     | Thermobifida alotholeras         | 2012  | ADV92525.1               | 29.8     | [15]       |
| Thc_Cut1    | Thermobifida cellulosilytica     | 2011  | ADV92526.1               | 29.4     | [16]       |
| Thc_Cut2    | Thermobifida cellulosilytica     | 2011  | ADV92527.1               | 29.7     | [16]       |
| TFCut2      | Thermobifida fusca               | 2015  | CBY05530.1               | 30       | [7]        |
| TFCut1      | Thermobifida fusca               | 2015  | CBY05529.1               | 30       | [7]        |
| BTA-2       | Thermobifida fusca               | 2015  | BAO42836.1               | -        | [7]        |
| Tfu-0883    | Thermobifida fusca YX            | 2010  | AAZ54920.1               | 29       | [9]        |
| Tfu-0882    | Thermobifida fusca YX            | 2015  | AAZ54920.1               | 29       | [9]        |
| Thf42_Cut1  | Thermobifida fusca               | 2011  | ADV92528.1               | 29.6     | [16]       |
| TFCa        | Thermobifida fusca KW3           | 2010  | CAZ65068.1               | 52.4     | [16]       |
| Tcur1278    | Thermomonospora curvat           | 2014  | ACY96861.1               | -        | [12]       |
| Tcur0390    | uncultured bacterium             | 2018  | ACY95991.1               | -        | [17]       |
| PET2        | Vibrio gazogenes                 | 2018  | ACC95208.1               | -        | [17]       |
| PET6        | Vibrio gazogenes                 | 2018  | SHF85073.1               | -        | [17]       |

3. Properties of cutinase

After FsC, many bacterial cutinase enzymes were discovered and characterized. Unlike the fungal cutinase, which is expressed in Pichia pastoris and Saccharomyces cerevisiae, most of the bacterial cutinase is expressed in E. coli, especially the BL21 strain. A large number of cutinase enzymes have been isolated and purified by various separation and purification methods such as affinity chromatography and ion exchange chromatography, and their biochemical properties have also been studied. The molecular weight of bacterial cutinase is slightly larger than that of fungal cutinase, generally 20-30kDa [12], and the molecular weight of TFCa is 52.4kDa. The optimum temperature for bacterial cutinase is 40-60°C, probably because they are all thermophilic sources. In general, cutinase works best at neutral or alkaline pH [14-16]. When pH is below 7, the enzyme activity drops sharply. But the optimum pH of Est119 is acidic, 6.0[10].

The cutinase substrate has a wide range, and most of them can hydrolyze various soluble esters, insoluble triglycerides, and natural polyester keratin. It is also possible to degrade polyesters such as polyethylene terephthalate (PET), polyacrylonitrile (PAN), nylon 6.6 and the like. For most bacterial cutinase, hydrolysis of p-nitrophenyl ester substrates conforms to the Michaelis-Menten equation and prefers short-chain substrates.

4. Structural study of cutinase

Studies on cutinase began with phytopathogenic fungi, and the cutinase from Fusarium solani is the most widely characterized. In contrast to true lipases, most cutinase enzymes do not cover the active site, resulting in exposure of the nucleophilic serine to the solvent [1]. The first X-ray crystal structure of the cutinase FsC from F. solani pisi was described in 1992[1,18]. Its structure shows that the F. solani pisi cutinase employs an α/β fold that catalyzes the exposure of serine (Ser120) to a solvent rather than using a hydrophobic loop to bury the active site. After FsC, many bacterial cutinase enzymes were discovered and characterized.

The cutinase belongs to the serine α/β hydrolase, the tertiary structure is highly homologous, and the catalytic triad is composed of S-H-D. The crystal structure determination of Est119 revealed that the overall structure showed a typical α/β-hydrolase fold [10,19]. It consists of a centrally twisted β-sheet
of nine β-strands with nine α-helices on each side. From the perspective of the 3D structure, the Est119 catalytic binding pocket is present at the same position as other serine esterases. In addition, a long surface groove extends from the catalytic bag.

5. Applications
Cutinase can hydrolyze soluble esters, insoluble triglycerides and various polyesters. In addition to hydrolysis, cutinase can also catalyze the esterification of acids with alcohols. Therefore, cutinase has many applications as a multifunctional enzyme.

5.1. Applications in the food field
Cutinase can be used for the hydrolysis of milk fat in the daily diet and the synthesis of triglycerides in the oleochemistry industry. The production of dehydrated fruit can accelerate the rate of dehydration by degrading the cuticle of the outer skin of the fruit, and contribute to the flavor and Infiltration of stabilizers [20]. Cutinase can be combined with proteases to remove proteins from tobacco.

Cutinase can be used for the synthesis of flavor esters such as butyl butyrate and ethyl hexanoate. Short-chain fatty acid esters are widely used in foods and medicines because of their pleasant fruity aroma. For example, butyl butyrate has apple, pineapple, banana and other fruit flavors, and can be applied to foods such as candy, biscuits, soda, ice cream and the like. Compared with the traditional chemical method, the enzymatic synthesis of flavor esters has mild reaction conditions, low energy consumption and environmental friendliness.

5.2. Plastic degradation applications
With its excellent properties such as durability, low gas permeability and transparency, polyester plastic has replaced many natural materials and has been widely used in various fields of human life. As a result, the output of plastic has increased more than 20 times over the past half century. At the same time as convenience, polyester plastic have created many problems that cannot be ignored. Most polyesters, including PET, are difficult to be degraded and tend to accumulate in the environment causing severe environmental damage. The chemical inertness of the aromatic compounds of PET makes the recycling process unfavorable. In addition, PET accumulates as a plastic waste in the ocean at a rate parallel to its level of production, which can cause environmental risks and negatively impact ecosystems. With the deepening of human understanding of environmental issues, biodegradable plastics have received much attention and become a hot topic of research. Cutinase can degrade PET, polycaprolactone (PCL), polybutylene succinate (PBS) and other synthetic polyesters [21], which have great application prospects in degrading plastics and environmental protection.

In addition, cutinase is capable of degrading phthalate plasticizers. Phthalates are POPs that are difficult to be degraded and easily enter the body through breathing, skin contact and eating contaminated food.

5.3. In other applications
Cutinase can degrade the plant stratum corneum and can therefore be used to extract active ingredients from plants. The use of cutinase can remove the properties of fat and is used as an enzyme for decomposing fat in detergents and as a detergent for dishwashing. The cotton scouring test using the combination of cutinase and pectinase showed that the cutinase can improve the wettability and whiteness of the fabric.

6. Conclusion and Prospects
Energy conservation and emission reduction are the focus of the current national macroeconomic regulation and control. As one of the key pollution sources in industrial system, the plastic industry has become the focus of attention. Among them, PET has been developed for use in the manufacture of polyester fibers and polymer films due to its excellent durability, low gas permeability and transparency, making it an attractive product in the textile industry and packaging market. Global plastic production
continued to grow in 2015, reaching 322 million tons [22], and PET accounted for about 7% of plastics demand. In 2011, domestic PET consumption was 26.336 million tons. In 2016, it reached 35.928 million tons. In 2016, the consumption of polyester fiber in China's PET consumption accounted for 76.6%; PET for bottle accounted for 17.4%; PET for film accounted for 5.0%; and other fields accounted for 1.0%.

Since most plastics are biodegradable but take a long time to be fully degraded, the amount of plastic waste expected to accumulate by 2050 will reach 33 billion tons [23, 24]. Currently only 14% of global plastic packaging materials are recycled, while another 14% are incinerated for energy recovery. The remaining 72% of the plastic packaging were not recycled, 40% of the land were filled, and 32% were estimated to have completely escaped from the collection system. This part of the plastic waste will eventually enter different natural habitats, especially in the ocean. A recent report by the Allen MacArthur Foundation warned that at least 8 million tons of plastic leaked into the ocean each year. Biocatalytic degradation can be applied as an eco-friendly method. Microorganisms decompose plastic by aggregating enzymes on the surface of the material to break the ester bonds.

So far, many cutinase enzymes have been found to effectively degrade PET [4, 12, 15, 25-28], which provides a basis for further exploration of plastic biodegradation, and also provides solutions to solve the increasingly serious problem of plastic pollution. However, the degradation rate of PET plastic currently found is still relatively low, the degradation time is long. There is a long distance from the actual application. Accordingly, to achieve high-efficiency biodegradation of PET plastic, the following key problems need to be solved: 1) the enzyme production level of the existing cutinase-producing bacteria is too low, the production cost of the enzyme is too high; 2) stability, especially thermal stability. It is a very important feature for cutinase in the degradation of PET. Plastic degradation needs to be carried out at above 65°C. So far, there are only a few thermostable cutinase enzymes, which come from thermophilic microorganisms such as T. fusca [6, 29]. However, not all cutinase from thermophilic sources has been proven to be thermostable. Therefore, it is necessary to screen and excavate cutinase with good thermal stability. At the same time, the thermal stability of the cutinase is improved by means of directional modification or immobilization.

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