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Review 综 述

微生物酯酶研究进展

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摘 要：酯酶自发现以来，逐渐被开发利用于医药、化工、食品等领域，其中动植物来源酯酶工业化应用较少，微生物作为天然的酶资源库，是新型酯酶的主要来源之一。然而，大量新型微生物酯酶由于活性低、稳定性差等原因难以达到工业应用的要求；同时酯酶的筛选、活性评价方法仍存在通用性低、成本高的问题，一定程度上阻碍了新型微生物酯酶挖掘和改造。据此，本文总结了近年来微生物酯酶分类与发现、结构与催化特性、改造和优化以及应用等领域的研究新进展，以期促进酯酶的挖掘和工业化应用。

关键词：微生物；酯酶；活性评价；结构优化

Research advances in microbial esterases

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Abstract: Esterases have been gradually developed and used in medicine, chemistry, and food industry since their discovery. Only a few esterases applied in industrial production are from animals and plants, and most of novel esterases are discovered from microorganisms, a vast enzyme resource pool in the nature. However, a large number of new microbial esterases do not

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meet the requirements of industrial application due to low activity and poor stability. Meanwhile, the mining of new microbial esterases is limited by low versatility and high cost of the screening and activity evaluation methods. This paper summarizes the recent research advances in the classification, discovery, structural and catalytic properties, modification, optimization, and application of microbial esterases, aiming to facilitate the discovery and industrial application of esterases.
Keywords: microorganism; esterase; activity evaluation; structure optimization

酯酶属于一种生物催化剂，能够催化短链脂肪酸与醇组成的酯发生酯水解、酯合成和酯交换反应，其广泛存在于动物、植物和微生物中[1]。动物酯酶分布于多种组织和器官中，在包括酯类药物和环境毒物在内的各种外源性物质代谢活动中发挥着重要作用,并参与机体的脂质稳态调节[2]；植物酯酶在植物的陆地定植演化、生殖发育和新陈代谢中有着重要的作用[3]；微生物酯酶与微生物生长繁殖、天然产物生物合成和毒力因子的代谢息息相关，在微生物的初级代谢、次级代谢和对环境中营养物质的利用过程中发挥着不可替代的作用。酯酶具有专一性强、底物谱广、反应条件温和、反应速率高以及无毒性等优势，凭借这些优势，酯酶尤其是微生物酯酶在食品、制药、纺织和造纸等领域发挥了不可替代的作用，也应用到生物聚合物和生物柴油的酶法合成中[4]。工业中使用的微生物酯酶能够通过工程化手段实现高效表达及大规模生产，且利用微生物酯酶生产产品具有能耗低、成本低、绿色环保等优点。随着微生物来源酯酶在工业生产及相关生物技术领域中的广泛应用，其相关研究也日益增多。

据此，本文阐述了近年来微生物酯酶在分类与发现、结构与催化特性、改造和优化以及应用等领域的新进展，以期为更好地开发和利用微生物酯酶资源提供理论参考。

1 微生物酯酶的分类与发现

酯酶通常指羧酸酯水解酶(carboxylic-ester hydrolases, EC 3.1.1)，其可分为羧酸酯酶(carboxylesterase, EC 3.1.1.1)、芳香酯酶(arylesterase, EC 3.1.1.2)、三酰基甘油脂肪酶(triacylglycerol lipase, EC 3.1.1.3)、磷脂酶 A2(phospholipase A2, EC 3.1.1.4)、溶血磷脂酶(lysophospholipase, EC 3.1.1.5)和乙酰酯酶(acetylesterase, EC 3.1.1.6)等。酯酶属于水解酶(hydrolases, EC 3)家族下的酯水解酶亚家族(ester hydrolases, EC 3.1)，该亚家族除羧酸酯水解酶外还包括硫酯水解酶(thioester hydrolases, EC 3.1.2)、磷酸单酯水解酶(phosphoric-monoester hydrolases, EC 3.1.3)和磷酸二酯水解酶(phosphoric-diester hydrolases, EC 3.1.4)等[5]。

酯酶中的三酰基甘油脂肪酶即常称的“脂肪酶”，一直以来，酯酶和脂肪酶的分类存在争议，在传统分类之外衍生出了许多分类方法，但并没有形成主流观点。如 Ali 等[6]从 4 个维度(物理化学、化学、解剖与组织、细胞)提出了一种脂肪酶和酯酶的分类标准(图 1)，该方法较为全面地考虑了 2 种酶的特质，酯酶与脂肪酶的区别主要集中在底物特异性和界面活化现象两方面，酯酶底物为水溶性的短链脂肪酸酯(<8 个碳原子)，酶促反应遵循米氏方程(Michaelis-Menten equation)，而脂肪酶底物为脂溶性长链脂肪酸酯(>8 个碳原子)，会在水-油界面产生界面活化现象[7]，酶促反应曲线为 S 型曲线。

目前已经存在诸多酯酶的活性测定方法，这些方法也促进了新型微生物酯酶的挖掘。微生物酯酶常用的测定方法包括分光光度法[8]和

荧光测定法[9]，也可以通过 HPLC、LC-MS 等方法检测降解产物来评价酯酶的活性[10]。分光光度法和荧光测定法检测各种酚类化合物(对硝基苯酚[8]、伞形酮[11]、荧光素[9]等)的显色基团或荧光基团产生的光谱变化，这些酚的颜色和荧光由酚阴离子的光谱特性产生，酚羟基在成酯后颜色和荧光被掩盖，而酯酶将酯键水解后产生游离酚重新激活显色基团或荧光基团，从而可以通过光谱方法定量测定。然而这些酚类化合物较低的 pKa (约为 7)使得酚酯底物在水中易水解，从而一定程度上降低了方法的可靠性，但通过化学修饰可以提高酚酯的稳定性，如 Reymond 等[12]在氟磷酸酯中插入乙酰氧基甲基提高了化学稳定性。

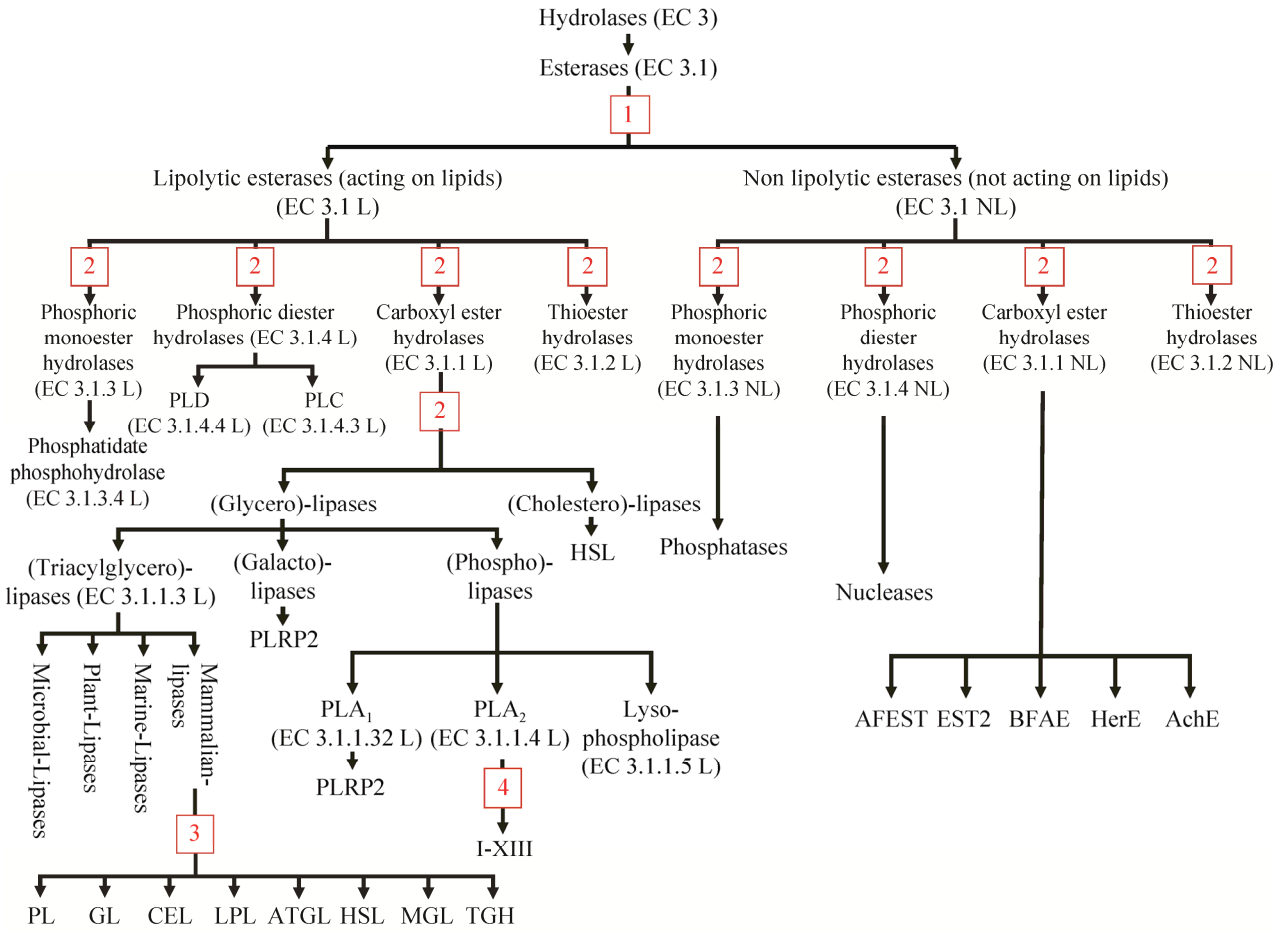

图 1　四重标准的酯酶分类[6]

Figure 1　Esterases classification according to four criteria[6]. 1: Physico-chemical; 2: Chemical; 3: Anatomical and tissular (only for mammalian (triacylglycero)-lipases); 4: Cellular (only for PLA2). Esterases can be divided into two main groups based on the physicochemical properties of their substrates: lipolytic (L) esterases (acting on lipids) and nonlipolytic (NL) esterases (not acting on lipids). AchE: Acetylcholinesterase; AFEST: *Archaeoglobus fulgidus* esterase; ATGL: Adipose triglyceride lipase; BFAE: Brefeldin A esterase; CEL: Carboxyl ester lipase; EST2: *Alicyclobacillus acidocaldarius* esterase; GL: Gastric lipase; HerE: Heroin esterase; HSL: Hormone-sensitive lipase; LPL: Lipoprotein lipase; MGL: Monoglyceride lipase; PLA1: Phospholipase A1; PLA2: Phospholipase A2; PLC: Phospholipase C; PLD: Phospholipase D; PL: Pancreatic lipase; TGH: TAG hydrolase.

上述常规活性评价方法的应用极大促进了微生物酯酶的筛选进程,在此基础上建立的酯酶高通量筛选方法具有易于操作、能够快速测定酶动力学参数和底物选择性以及精度和重复性高等优点[13]。Ramírez 等[14]报道了一种基于非显色底物 4-硝基苯酚的 pH 指示剂 96 微孔板筛选方法，用于同时测定阿魏酸酯酶、单宁酶和氯代酯酶的活性；Lusty Beech 等[15]使用磺基酞酸酯染料，在 96 微孔板中筛选得到了 9 种能够降解聚对苯二甲酸乙二醇酯(bis-(2-hydroxyethyl) terephthalate，BHET)的酯酶。Bhargava 等[16]开发了高通量、基于荧光的酯酶活性测定方法，用于评估生物制药过程中聚山梨醇酯降解程度，可在 3 h 内快速测定酯酶活性。高通量筛选方法也可以利用手性或前手性底物快速筛选手性酯酶，以天冬氨酸营养缺陷型大肠杆菌(*Escherichia coli*)为宿主菌株，对酯酶的伯醇外消旋酯对映选择性进行筛选，将突变文库接种在含有所需伯醇对映体的天冬氨酸酯的培养基上，通过添加特定对映体膦酸酯抑制对映选择性较低的菌株生长，该膦酸酯能共价抑制对映选择性较低的酶变体，因此只有能够水解特定对映体的菌株才能利用天冬氨酸进而满足其营养需求，而偏好相反对映体的菌株生长受到抑制[17]。Böttcher 等[18]建立了一种基于微量滴定板的检测方法，该方法先使用琼脂平板覆盖测定法对活性和非活性单克隆进行鉴别，然后在微量滴定板中用次级醇的醋酸盐作为底物测定酯酶的活性和对映选择性，每天可筛选数千个酯酶突变体。

获取微生物酯酶一般需要微生物的纯培养，在培养基中添加底物，随后通过提取和分析来评价酯酶活性，这种方法通常比较耗时且会发现大量重复酯酶。Marmuse 等[19]通过化学-酶法合成吲哚硝基苯-5-O-羟基肉桂酰-α-L-阿拉伯呋喃糖苷用于阿魏酸酯酶的活性评价，首先用琼脂平板筛选以去除无活性的酯酶，再检测其余酯酶的活性。Alberto 等[20]开发了高通量、高可靠性、高重复性的培养方法筛选能够表达酯酶的真菌转化子,使用自动微型细胞培养仪和微孔板筛选转化子,每天可以筛选 800 个克隆。但环境中的绝大多数微生物都不能在实验室实现纯培养，这使得大量的微生物资源难以得到开发和利用,随着下一代测序技术的发展,人们通过宏基因组学开发微生物资源，这一想法也应用到了新型酯酶的挖掘中。宏基因组学能够通过测序获得环境中未培养微生物的大量遗传信息，构建宏基因组文库后通过功能筛选和序列分析挖掘新型酯酶。如 Lee 等[21]构建了牛瘤胃宏基因组文库，通过功能筛选进行初筛后，结合序列分析得到了能够降解氯吡硫磷的新型酯酶 Est3S；Park 等[22]从堆肥宏基因组库中分离和鉴定了 2 个新型酯酶基因 *est8L* 和 *est13L*。但宏基因组文库筛选很大程度上取决于蛋白在异源宿主中能否正确转录、翻译和折叠，使用大肠杆菌作为宿主时，通过功能性宏基因组筛选只能从宏基因组文库中回收一部分蛋白质，开发适用性更广的蛋白表达宿主有助于解决这一问题[23]。

近年来,越来越多的科学家开始关注极端环境微生物来源的酯酶，如嗜冷[24]、嗜热[25]和嗜盐[26]性质的酯酶；在极端环境下发现的酯酶可作为在恶劣和非常规条件下使用的生物催化剂。

2 微生物酯酶的结构和催化特性

大部分微生物酯酶属于 α/β 水解酶超家族，含有 α/β 水解酶的由 8 条基本平行的 β 折叠组成中心 β 片和周围有 5 条围绕蛋白质核心的 α 螺旋的典型折叠结构。许多 α/β-水解酶 N 端 α1 螺旋和 α2 螺旋之间的无规卷曲形成了结构高

度可变的帽状结构域，其通常位于水解酶结构域中活性位点的顶部[27]。目前已有大量微生物酯酶的三维结构被解析，这些酶大多表现出了相似的核心拓扑结构，二级结构中的 α 螺旋和 β 折叠由柔性环连接。酯酶通常都具有一个“催化三元组”即 Ser-His-Asp/Glu，这些氨基酸残基在序列上高度保守，蛋白质正确折叠后 3 个残基在空间上聚集，形成 α/β 水解酶核心结构域的活性位点而行使功能[28]。“催化三元组”的 Ser-His-Asp/Glu 形成亲核-碱-酸的电荷中继系统，其中的丝氨酸常位于 β5 折叠后的急转弯处。“催化三元组”大致位于酶中心底物口袋的底部，距离酶表面约 20–25 Å，由活性丝氨酸一侧的大柔性口袋和另一侧的小刚性口袋组成。活性位点的方向和位置为各种底物的水解提供了理想的环境[29]。此外，微生物酯酶活性位点附近还存在氧阴离子空穴，由 α3 螺旋、β3 折叠和 β5 折叠围成，它能够稳定蛋白质的三级结构[30]。图 2 展示了具有典型微生物酯酶结构的荧光假单胞菌(*Pseudomonas fluorescens*)[31]和嗜热地芽孢杆菌(*Geobacillus stearothermophilus*)[25]来源酯酶的结构。

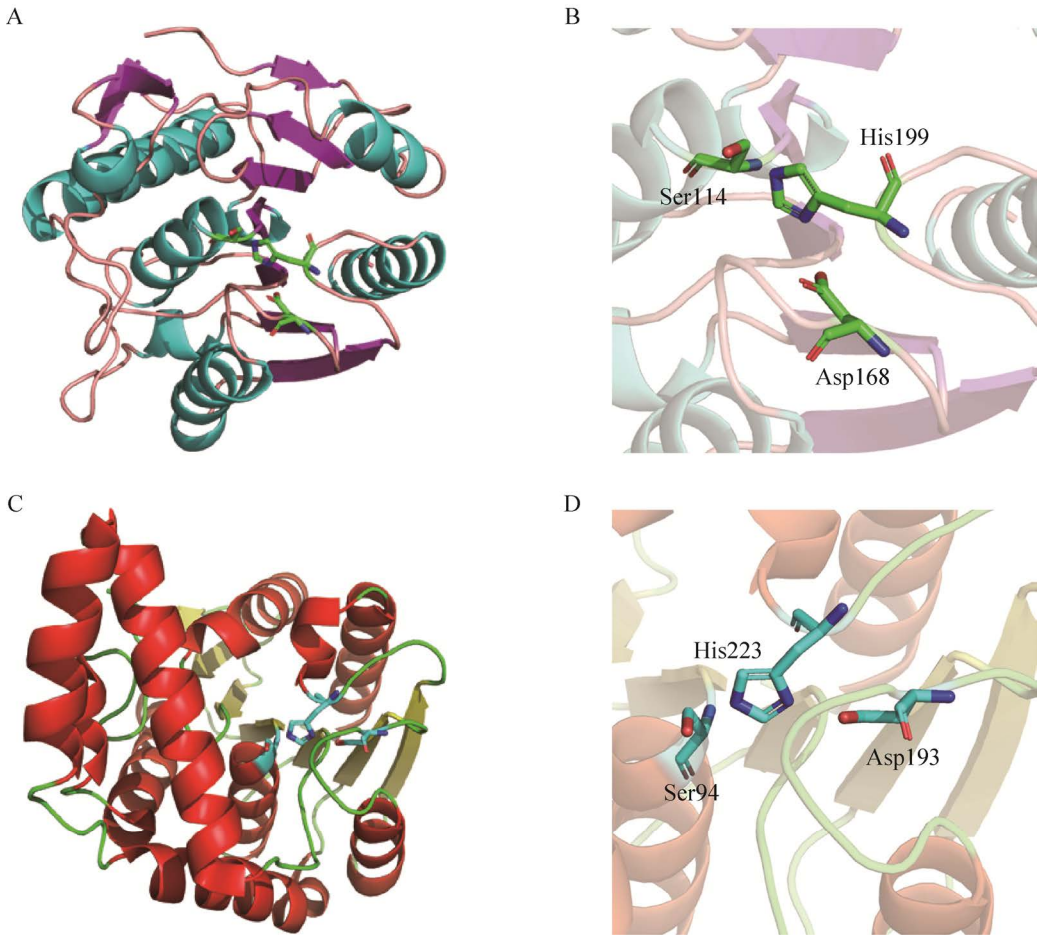

图 2　荧光假单胞菌和嗜热地芽孢杆菌酯酶的晶体结构[25,31]

Figure 2　Crystal structures of *Pseudomonas fluorescens* and *Geobacillus thermophilus* esterases[25,31]. A–B: The structure of esterase from *Pseudomonas fluorescens* (PDB ID: 1AUO). C–D: The structure of esterase from *Geobacillus stearothermophilus* (PDB ID: 1TQH), the catalytic active sites are annotated.

酯酶能够催化酯键水解、形成以及酯交换反应(图 3)，其因反应条件温和、稳定性良好、化学选择性和立体选择性高而被广泛用于生物催化。酯酶的经典催化机制包括 2 步，第一步是丝氨酸羟基对底物羰基的亲核攻击，“催化三联体”的组氨酸和天冬氨酸形成氢键，丝氨酸羟基向组氨酸给出 1 个质子形成“氧阴离子空穴”，此时丝氨酸由附近的 2 个甘氨酸稳定，形成瞬态四面体中间体，丝氨酸羟基的氧负离子进攻底物的羰基基团，底物的一部分与先前质子结合形成醇，另一部分形成丝氨酸-酰基复合物；第二步由组氨酸激活 1 个水分子，水分子亲核攻击丝氨酸-酰基复合物的羰基将其水解，同时组氨酸向丝氨酸提供质子，释放底物的酰基部分形成酸[27]。本实验室前期对链霉菌(*Streptomyces* sp.) 139 胞外多糖依博素生物合成基因簇的研究发现，阻断调控基因 *ste1* 和 *ste2* 后，依博素的产量均有显著提高[32-33]；调控基因 *ste1* 和 *ste2* 阻断株的转录组测序结果表明，调控基因阻断后依博素生物合成基因簇中 *orf2* 基因的转录水平显著提高，生物信息学预测结果表明该基因编码肽酶 E 家族(dipeptidase E, EC 3.4.13.21)蛋白。按照常规功能预测，该家族的蛋白能够降解 N 端为天冬氨酸的二肽(Asp-X)[34]；但在实际研究中却初步发现 Orf2 能够水解对硝基苯酸酯的酯键，有关此酶的酶学性质及其与依博素生物合成的相关性正在进一步研究中。

3 微生物酯酶的改造和优化

3.1 微生物酯酶蛋白质工程

利用定向进化和理性设计等蛋白质工程方法改善酯酶的性质，一直是酯酶作为高效生物催化剂发展的主要途径[35]。用于深入分析酶催化机制(包括立体选择性)补充技术(如生物信息学、蛋白质晶体学、光谱方法)的快速发展，结合理性设计等蛋白质工程技术使得酯酶工程化改造成为可能[36]。蛋白质工程不仅能改善酯酶作为生物催化剂的性质，还有助于更好地理解蛋白-底物相互作用的机制。定向进化着眼于改善蛋白质的性质而不论突变位点，因此需要构建较大的随机突变文库并进行筛选。目前，对蛋白质结构与功能的关系理解不足，难以预测能够影响蛋白性质的突变位点，因此一些生物信息学工具被开发出来用以预测突变对蛋白性质的影响[37]。Shin 等[38]对微生物来源的酯酶 Est3 进行理性设计，将活性位点外的长 loop 区替换为短 loop 区，使该酶对对硝基苯丁酸酯和

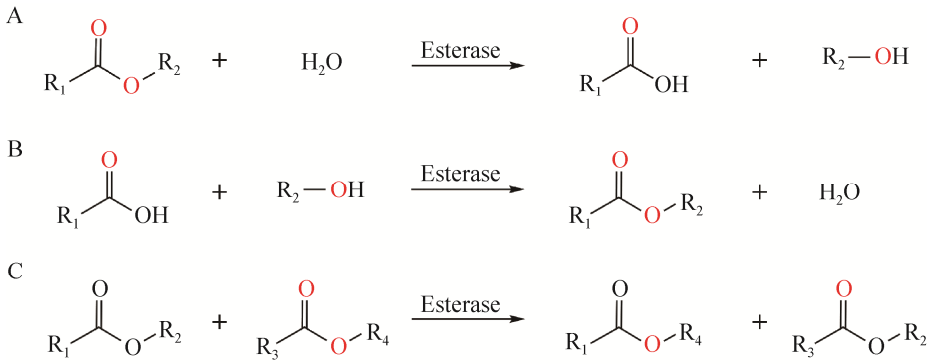

图 3 酯酶催化反应

Figure 3 Esterase-catalyzed reaction. A: Ester synthesis reaction. B: Ester hydrolysis reaction. C: Ester exchange reaction.

对硝基苯棕榈酸酯的水解活性分别增加了4.3倍和 4.6 倍。Li 等[39]使用结构导向的蛋白质工程对名为 CrmE10 的微生物酯酶进行改造，将蛋白质的 5 个酸性氨基酸残基突变为碱性氨基酸残基，提高了该酶在碱性条件下的活力，在 pH 10.5 时酶活性从 0 提高到 20%。蛋白质的理性设计仅适用于已知具有潜在功能的氨基酸残基，从而排除了对活性和稳定性具有负面影响的突变位点。半理性设计是定向进化和理性设计之间的一个折中方案，其结合了定向进化和理性设计的优点，根据已有研究和序列比对结果对活性、稳定性和对映选择性至关重要的特定氨基酸残基进行饱和突变，建立小型突变文库，并对其进行筛选[40]。通过这些改造可以有效改善酯酶性质，并有助于了解其作用机制。

3.2 微生物酯酶固定化和表面展示

酶固定化是一种改善酶学性质的通用方法，酶固定化后易于回收，稳定性高，同时部分酶的选择性也能得到改善[41]。酯酶固定化后可以显著改善嗜热酯酶的性质[42]，嗜热脂肪芽孢杆菌(*Bacillus stearothermophilus*)来源嗜热酯酶通过多点共价连接固定在乙醛基琼脂糖凝胶上，提高了酯酶的稳定性，在 50% N,N-二甲基甲酰胺(DMF)或二甲硫醚(DMS)中 30 °C 放置 1 周后仍能保留 65%的活性[43]，其对酯酶的体外生物合成相关研究具有较大意义。

酶的表面展示技术能够利用高催化活性的酶构建全细胞催化剂，将酶以共价或非共价的方式固定于细胞外表面，相比固定化酶免去了载体制备和蛋白纯化的步骤。Liu 等[44]使用表面展示技术在大肠杆菌中表达了红霉素酯酶，该工程菌株在体外能够降解红霉素，将该菌株注射至小鼠肠道之后，小鼠粪便中的红霉素显著减少。

3.3 微生物酯酶的反应条件优化

酯酶的生物催化会受到很多因素的影响，如底物、溶剂、无机盐、表面活性剂、底物/酶浓度、温度和 pH 等。如来源于骆驼瘤胃宏基因组的酯酶 Est-CR，在 K^{2+}、Ca^{2+}、Mg^{2+}和 Mn^{2+}的存在下活性提高[45]。来源于地衣芽孢杆菌(*Bacillus licheniformis*) B-1 的酯酶在适当浓度的 Zn^{2+}、Mn^{2+}、Mg^{2+}、吐温 80、SDS、Triton X-100 和 BSA 存在下，酶活性大大提高[46]。来源于堆肥宏基因组的 Est2，在 6%−30% (体积分数)的甲醇、乙醇、正丙醇、异丙醇存在下活性显著增强了 2−10 倍[47]。因此对上述这些反应条件的优化能够极大地改善酶的活性甚至是立体选择性[48]，这对于提高酯酶性能，了解酯酶结构-功能关系具有重要意义。

4 微生物酯酶的应用

微生物来源的酯酶已经在生活中得到了广泛应用，且近年来应用愈加广泛，表 1 列出了近年来一些新型酯酶的应用现状。

4.1 微生物酯酶在工业合成中的应用

酯酶可广泛用于手性醇(伯醇、仲醇和叔醇)酯的对映选择性水解和拆分，来自枯草芽孢杆菌(*Bacillus subtilis*)的萘普生酯酶能够催化萘普生甲酯的对映选择性水解以产生 S-萘普生[75]。Liu 等[76]报道了酶法拆分 2,2-二甲基环丙烷羧酸(DMCPA)外消旋酯，它能够产生(S)-DMCPA 作为西司他丁合成中间体。有机化学中叔醇酯在温和条件下的水解有较大价值，尤其是在手性醇拆分和去除羧基保护基方面。α/β 水解酶的几个亚类具有更宽泛的活性位点，在氧阴离子空穴中含有 3 个甘氨酸的保守位点，属于 GGG(A)X 家族的酯酶能够催化叔醇酯的酶水解，并已得到商业化应用[77]。在枯草芽孢杆菌 BCL1050 中使用重组萘普生酯酶(其活性较野

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表 1　近年来部分新发现酯酶及其应用

Table 1　Newly discovered esterases and their applications in recent years

Name	Optimum pH	Optimum temperature	Source	Application	Year	Reference
			Paenarthrobacter sp. strain Shss	Degradation of phthalates	2022	[49]
FeaB			*Sphingopyxis fribergensis* Kp5.2	Biosynthesis of phenylacetic acid	2022	[50]
EreC			*Klebsiella pneumoniae* 05-506	Degradation of macrolide antibiotics	2021	[51]
FaeLam			*Lactobacillus amylovorus* CGMCC11056	Assist in protein secretion	2021	[52]
LF18			*Lactobacillus farciminis* LF18	Improving fermentation quality of *Sorghum Silage*	2021	[53]
LP23			*Lactobacillus plantarum* LP23	Improving fermentation quality of *Sorghum Silage*	2021	[53]
	8.0	60 ℃	*Enterobacter mori* BI1245	Degradation of phthalates	2021	[54]
			Bacteroides intestinalis	Degradation of complex structural xylan	2021	[55]
PfeE			*Pseudomonas aeruginosa* PAO1	Involved in the biological process competing for iron	2021	[56]
Hyd			*Rhodococcus* sp. 2G	Degradation of phthalates	2020	[57]
EreA	7.0	35 ℃	*Delftia lacustris* RJJ-61	Degradation of erythromycin in fermentation waste	2020	[58]
Est3			*Sphingobium chungbukense* DJ77	Degradation of polycaprolactone	2020	[38]
Pxpme	5.0	45 ℃	*Paenibacillus xylanexedens* SZ 29	Degradation of pectin during food processing	2020	[59]
EstCS3			Metagenomic library of compost	Degradation of ampicillin, cefotaxime and cefepime	2020	[60]
LanE			*Edaphocola flava* HME-24	Degradation of lactofopp	2020	[61]
XtjR8	8.0	40 ℃	Metagenomic library of lotus pond sludge	Degradation of phthalates	2020	[62]
			Pseudomonas nitroreducens CW7	Degradation of various pesticides such as allethrin	2020	[63]
EstGSU753	8.0	60 ℃	*Geobacillus subterraneus* DSM13552	Production of short-chain flavor esters	2020	[64]
EstB	8.0	40 ℃	*Enterobacter* sp. HY1	Degradation of bis(2-hydroxyethyl) terephthalate	2020	[65]
BpFae12	8.0	50 ℃	*Bacillus pumilus* W3	Degradation of rosmarinic acid and chlorogenic acid	2020	[66]
			Candida parapsilosis	Production of short-chain flavor esters	2020	[67]
DpeH			*Microbacterium* sp. PAE-1	Degradation of phthalates	2020	[68]
	6.5	45 ℃	*Lactobacillus fermentum* LF-12	Degradation of dicaffeoylquinic acid	2020	[69]
EstM2	8.0	37 ℃	Metagenomic library of soil	Degradation of phthalates	2020	[70]
EstCS1	8.0	50 ℃	Metagenomic library of compost	Degradation of tertiary alcohol esters	2020	[71]
EstZY	9.0	50 ℃	*Alicyclobacillus tengchongensis*	Removal of 7-aminocephalosporanic acid (7-ACA) C3' acetyl group	2020	[72]
BaCEs04	7.5	60 ℃	*Bacillus velezensis* SYBC H47	Degradation of phthalates	2020	[73]
DmtH	7.6	30 ℃	*Sphingobium* sp. C3	Degradation of dimethyl terephthalate	2020	[74]

生型菌株高 800 倍)显著提高了(S)-萘普生的产量。反应条件优化后，150 g/L 外消旋萘普生甲酯转化率为 45.1%，且(S)-萘普生的占比为 99%[78]。阿魏酸酯酶水解底物释放的羟基肉桂酸由于其抗氧化性能被广泛用于化妆品和制药工业[79]，阿魏酸酯酶还可用于纸浆和纸张加工业、作为动物饲料添加剂促进养分同化[80]，以及促进木质纤维素生物质转化为可发酵糖用于生产生物乙醇[81]。此外阿魏酸酯酶还用于香精酯和人工甘油三酯等的制备，以及水和土壤中各种杀虫剂的检测[82]。

4.2 微生物酯酶在食品加工中的应用

食品加工方面，许多食品的生产过程需要微生物参与，而酯酶在微生物参与的食品加工过程中起到了重要作用。如在白酒和醋的生产过程中[83]，微生物酯酶能够影响大曲的风味。酯酶纯化后，可用于风味酯如乙酸乙酯、乙酸肉桂酯、香精酯、乙酸正丙酯、乙酸异丁酯、乙酸异戊酯的合成[64,67]。奶酪的生产过程中，酯酶将牛奶三酰甘油脂分解为各类游离脂肪酸，使奶酪产生了独特的风味[84]。Zhong 等[59]发现了一种果胶酶 Pxpme，其能够显著提高菠萝块的硬度(114%)，可以用于食品加工。此外，酯酶还可以提高高粱青贮的发酵品质[53]。

4.3 微生物酯酶在生物降解中的应用

各类农药、抗生素、塑化剂的广泛应用对环境造成了污染，常规的物理、化学方法处理这些污染物不仅成本高昂，还可能产生二次污染，而酯酶能够高效且环保的降解环境中这些污染物，如乳氟禾草灵[61]、烯丙菊酯[63]、氯吡硫磷[21]、大环内酯类抗生素[51,58]、β-内酰胺类抗生素[60,72]、邻苯二甲酸酯、聚己内酯等，酯酶在污染物的生物降解领域具有较大的应用前景。

5 展望

本文综述了微生物来源酯酶在结构、分类、催化机制、筛选、改造、优化和应用等方面的研究进展。目前已有大量微生物来源新型酯酶被报道，但只有少部分得以应用到工业生产中。许多微生物酯酶由于活性低、稳定性差等特性难以在工业中得到实际应用，即使经过改造也难以达到工业应用的要求；同时酯酶的筛选和活性评价方法仍存在通用性低、操作繁琐、成本高的问题，在挖掘降解特定底物的酯酶时存在较大困难。要解决以上的问题需要不断探索新的酯酶筛选及活性评价方法，同时研究酯酶的酶促反应机制，解析酯酶结构-功能之间的关系，并结合蛋白质工程的定向进化、理性设计和半理性设计等技术对酯酶进行改造，促进微生物酯酶在制药、化学合成、生物质降解、食品和饲料工业、服装、农用化学品、生物修复和生物传感器开发等领域的应用，使微生物酯酶相关研究更好地服务于诸多行业。

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