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Review 综 述

裂解性多糖单加氧酶及其应用研究进展

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摘　要：裂解多糖单加氧酶(lytic polysaccharide monooxygenases, LPMOs)是近几年新发现的氧化酶，该酶在生物质酶解方面发挥着重要的作用，因此，被描述为生物质解构助推器。LPMOs与底物的结合具有特异性，催化机理尚未完全阐明。虽然关于LPMOs的研究很多，但真正投入到工业生物质转化中的却很少，这对它们的表达、调控和应用都提出了挑战。本文首先系统综述了LPMOs的发现与分类、催化机制、构效关系，其次探讨了LPMOs的活性测定方法及重组表达技术，最后协同综述了LPMOs在不同领域的应用并对未来的研究方向进行了展望。本综述有助于加深对LPMOs的系统认识，推动LPMOs及其酶工程的研究，以期为LPMOs的研究和应用提供参考。

关键词：裂解多糖单加氧酶；生物质转化；生物催化；生物能源；纳米纤维

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Lytic polysaccharide monooxygenase and its application

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Abstract: Lytic polysaccharide monooxygenases (LPMOs) are newly discovered copper ion-dependent oxidases, which play an important role in the enzymatic hydrolysis of biomass. Therefore, LPMOs have been described as biomass deconstruction boosters. LPMOs bind to specific substrates, and their catalytic mechanism has not been fully elucidated. Although there are many studies involving LPMOs, few of LPMOs have been applied to industrial biomass conversion, which poses challenges for their expression, regulation, and application. We comprehensively review the recent advances in LPMOs from the aspects of discovery and classification, catalytic mechanism, and relationship between structure and function. Further, we systematically summarize the activity determination and recombinant expression methods of LPMOs. Finally, we introduce the applications of LPMOs in different fields and put forward the future research directions. This review helps deepen the systematic understanding and promote the research and engineering of LPMOs, which will provide reference for the application of LPMOs.

Keywords: lytic polysaccharide monooxygenases; biomass conversion; biocatalysis; biological energy; nano-cellulose

生物质能作为仅次于煤炭、石油、天然气的第四大能源，在应对全球气候变化、环境污染和能源短缺方面潜力巨大[1]。其中，木质纤维素现存量最大，在转化为绿色生物燃料和增值化学原料方面具有广阔的应用前景，其高值化和资源化利用对维持全球碳循环和生物炼制产业的发展具有重要作用[2]，也是我国“碳达峰、碳中和”行动方案中的重要内容。木质纤维素转化主要包括预处理、酶解糖化以及发酵 3 个步骤，其中木质纤维素生物转化可行性的决定性因素主要是酶解糖化[3]。由此可见，高效纤维素降解酶的创制面向国家重大需求，具有广阔的市场前景。

如图 1 所示，木质纤维素由纤维素、半纤维素和木质素组成，其中，纤维素是由纤维二糖单元组成的糖链通过氢键作用而形成的聚合物，其复杂的结构制约着纤维素酶促水解的效率。早期的研究以纤维二糖水解酶(cellobiohydrolase, CBH)、内切葡聚糖酶(endoglucanase, EG)和 β-葡糖苷酶(β-glucosidase, BGL)对纤维素进行协同酶法降解，以实现木质纤维素的高效生物质转化[4]。长期以来，本课题组致力于纤维素发酵产醇酿酒酵母菌株的构建及工程化改造研究，在酿酒酵母中首创了 POT1 介导的 δ 整合方法，利用此方法实现了 CBH 在酿酒酵母中的高拷贝、稳定表达，所构建菌株 SK8-5 的酶活性高达 238 mU/g，是目前已知报道的最高酶活[5]；此外还构建了一批含有不同纤维素酶比例的适用于不同材质纤维素降解的酵母菌株，并从多角度多层次对菌株进行了工程化改造，但研究

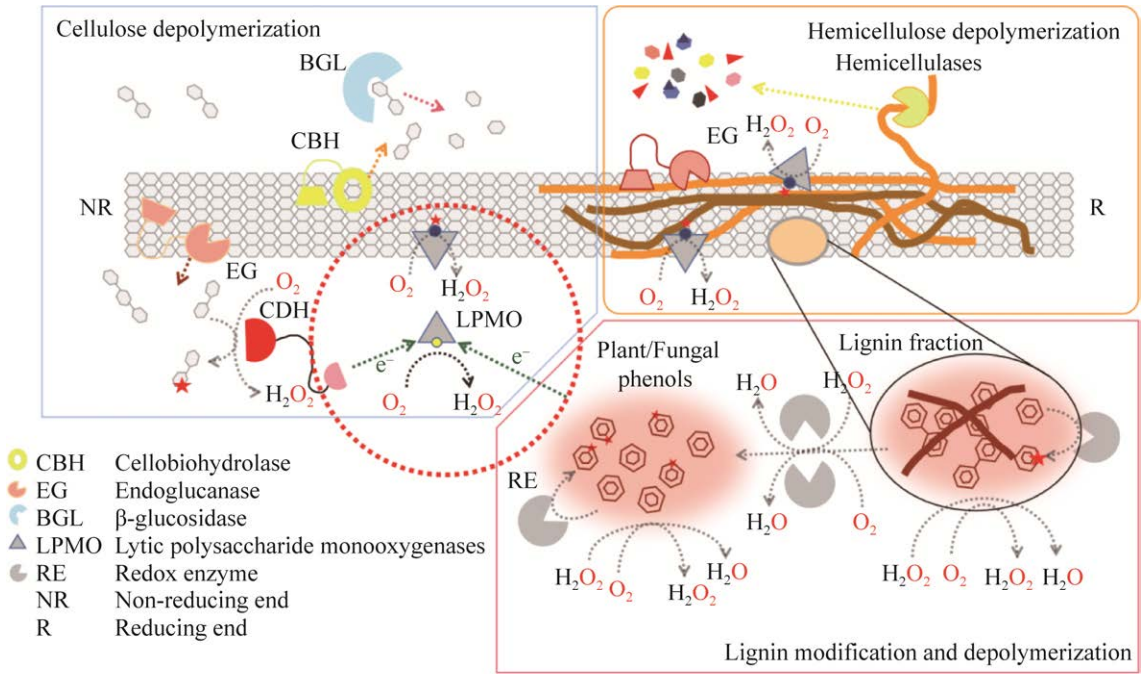

图 1　裂解多糖单加氧酶(lytic polysaccharide monooxygenases, LPMOs)在木质纤维素降解中的作用示意图
Figure 1　Schematic diagram of the role of lytic polysaccharide monooxygenases (LPMOs) in lignocellulose degradation.

发现制约生物质高效转化的瓶颈主要是纤维素的低酶解效率[6]。近几年来的研究发现，裂解多糖单加氧酶(lytic polysaccharide monooxygenases, LPMOs)可通过氧化裂解破坏纤维素的结构，对纤维素的高效酶解起到重要作用，是极具开发潜力的纤维素降解酶，这一新酶的发现为木质纤维素的酶法降解开辟了新的道路[7-9]。本课题组在前期工作中也初步证实了在 *Ta*LPMO 的协同作用下，糖苷水解酶可大幅度提高纤维素的降解效率。

因此，本文对 LPMOs 的最新研究进展进行综述，包括 LPMOs 的发现与分类、催化机制、构效关系、活性测定方法、重组表达技术及 LPMOs 在不同领域的应用，以期为深入开展 LPMOs 及其生物工程应用研究提供指导。

1　LPMOs 的发现与分类

LPMOs 在自然界中分布广泛。2008 年，Karkehabadi 等[10]发现了糖苷水解酶 61 (glycoside hydrolase 61, GH61)与传统 GH 家族之间的结构差异，标志着 GH61 家族的兴起。2011 年，Quinlan 等[11]证实 GH61 家族含有 LPMOs，该酶可通过氧化裂解糖苷键。2013 年，LPMOs 归类于 carbohydrate-active enzymes database (CAZy)数据中的辅助活性蛋白家族，LPMOs 可实现难降解多糖的转化，这引起广大学者的极大关注[12]。

如表 1 所示，目前所报道的 AA9 家族(之前被称为 GH61)都来自真菌，具有纤维素裂解活性[10-11,13-31]。AA10 家族(之前被归类到 CBM33)主要来源于细菌和放线菌，具有纤维素裂解活性，少数具有几丁质裂解活性[30,32-41]。自然界中发现的 AA11 和 AA13–AA17 家族的 LPMOs 蛋白数量相对较少，一般都是从真菌中提取的，这些真菌中的大多数也能产生降解其他多糖的酶，所以这些菌株可能是新型 LPMOs 的有趣来

表 1 LPMOs 的来源、结构和底物

Table 1 The source, structure and substrate of LPMOs

CAZy class	Enzyme	PDB code	Source organism	Known substrate(s)	References
AA9	*Pc*LPMO9D	4B5Q	*Phanerochaete chrysosporium*	PASC, Avicel	[13]
	AN3046	–	*Aspergillus nidulans*	PASC, xyloglucan	[14]
	*Mt*LPMO3	5UFV	*Myceliophthora thermophila*	PASC	[15]
	*Mt*LPMO9L	–	*M. thermophila*	PASC, Avicel	[16]
	GH61E	3EJA	*Thielavia terrestris*	PASC, Avicel	[17]
	*Hi*LPMO9B	5NNS	*Heterobasidion irregulare*	Cellulose	[18]
	*Hi*LPMO9H	–	*H. irregulare*	PASC	[19]
	*Nc*LPMO9C	4D7U	*Neurospora crassa*	Cellulose, hemicellulose, soluble oligosaccharide	[20-21]
	AN1602	–	*A. nidulans*	PASC, cellohexaose	[14,22]
	*Nc*LPMO9D	4EIR	*N. crassa*	PASC	[23]
	*Ls*AA9A	5ACF	*Lentinus similis*	Cellulose, xyloglucan, glucan, glucomannan, soluble, oligosaccharides	[24]
	*Nc*LPMO9A	5FOH	*N. crassa*	Cellulose, PASC, xyloglucan, glucomannan	[25]
	*Ta*LPMO9A	2YET	*Thermoascus aurantiacus*	PASC	[11]
	*Gt*LPMO9A	–	*Gloeophyllum trabeum*	PASC, CMC, xyloglucan, glucomannan	[26]
	GtLPMO9B	–	*G. trabeum*	PASC, xyloglucan	[27]
	*Hj*LPMO9A	5O2W	*Hypocrea jecorina*	Cellulose, PASC	[28]
	*Hj*LPMO9B	2VTC	*H. jecorina*	Cellulose	[10]
	*Mt*LPMO9A	–	*M. thermophila*	Avicel, PASC, xyloglucan, xylan	[16,24,29-31]
	*Cv*AA9A	5NLT	*Collariella virescens*	Cellulose, xyloglucan, glucan and glucomannan, soluble oligosaccharides	[24]
AA10	*Ba*AA10A	2YOW	*Bacillus amyloliquefaciens*	α and β chitin	[30]
	*Cj*LPMO10A	5FJQ	*Cellvibrio japonicus*	α and β chitin	[32]
	*Bl*LPMO10A	5LW4	*Bacillus licheniformis*	α and β chitin	[33]
	*Jd*LPMO10A	5AA7	*Jonesia denitrifcans*	α and β chitin	[34]
	*Sm*AA10A	2BEM	*S. marcescens*	α and β chitin	[35]
	*Sc*AA10C	4OY7	*Streptomyces coelicolor*	PASC, Avicel, β chitin	[36]
	*Sli*LPMO10E	5FTZ	*Streptomyces lividans*	β chitin	[37]
	*Sam*LPMO10B	–	*Streptomyces ambofaciens*	β chitin	[38]
	*Sam*LPMO10C	–	*S. ambofaciens*	PASC, flax pulp fibers	[38]
	*Tf*AA10B	–	*Thermobifida fusca*	PASC Avicel	[39]
	*Na*LPMO10A	–	*Natrialbaceae archaeon*	Chitin	[40]
	*Tt*AA10A	6RW7	*Teredinibacter turnerae*	Cellulose	[41]
	*Tf*AA10A	4GBO	*T. fusca*	Chitin, PASC, Avicel	[39]
	*Sc*LPMO10B	4OY6	*S. coelicolor*	Cellulose, chitin, PASC, Avicel	[36]
AA11	*Ao*AA11	4MAI	*Aspergillus oryzae*	β chitin	[42]
	*Ff*AA11	–	*Fusarium fujikuroi*	α and β chitin, lobster shells	[43]

(待续)

(续表 1)

CAZy class	Enzyme	PDB code	Source organism	Known substrate(s)	References
AA13	*An*AA13	–	*Aspergillus nidulans*	Starch	[44]
	*Nc*AA13	–	*N. crassa*	Amylose, amylopectin, cornstarch	[45-46]
	*Mt*AA13	–	*M. thermophila*	Amylose, amylopectin, cornstarch	[46]
	*Ao*AA13	4OPB	*A. oryzae*	–	[44,47]
	*At*LPMO13A	–	*Aspergillus terreus*	Wheat starch	[48]
	*Mo*LPMO13A	–	*Magnaporthe oryzae*	Binding to wheat starch	[48]
AA14	*Pc*AA14A	–	*Pycnoporus coccineus*	Xylan	[49]
	*Pc*AA14B	5NO7	*P. coccineus*	Xylan	[49]
AA15	*Td*AA15A	5MSZ	*Thermobia domestica*	Avicel, β chitin	[50]
	*Td*AA15B	–	*T. domestica*	α and β chitin	[50]
	*Dm*AA15A	–	*Drosophila melanogaster*	–	[50]
	*Dm*AA15B	–	*D. melanogaster*	–	[50]
	*Tc*LPMO15-1	–	*Tribolium castaneum*	–	[51]
	*Lm*LPMO15-1	–	*Locusta migratoria*	–	[51]
	*rOf*LPMO15-1	–	*Ostrinia furnacalis*	β chitin	[51]
AA16	*Aa*AA16	–	*Aspergillus aculeatus*	PASC, nano-fibrillated cellulose	[52]
AA17	*Pi*AA17A	–	*Phytophthora infestans*	Pectin	[53]
	*Pi*AA17B	–	*P. infestans*	Pectin	[53]
	*Pi*AA17C	6Z5Y	*P. infestans*	Pectin	[53]

–: No data.

源[42-53]。还有一些 LPMOs 的来源菌株是攻击植物的病原体[54]，这些菌株中的 LPMOs 在降解植物多糖中发挥作用。AA15 来源于热蝇和黑腹果蝇，参与昆虫多糖的代谢，并影响它们的生理发育[50-51]。

2 LPMOs 的催化机制

随着对 LPMOs 研究的不断深入，其催化机理得到了广泛的研究，特别是其共底物是 O_2 还是 H_2O_2 的问题引起了更多的讨论。早期的研究发现，LPMOs 的催化过程依赖于 O_2 的参与。在还原剂作用下，活性中心的 Cu^{2+}被还原为 Cu^{+}，可以特异性氧化多糖的 C1 或 C4 位碳原子，或同时氧化 C1 和 C4 位碳原子[7]。后来的研究发现，未与底物结合的 LPMOs 可将 O_2 转化为 H_2O_2[55]，表明 H_2O_2 是 LPMOs 反应的首选共底物而不是 O_2。此外，研究还表明依赖于 H_2O_2 的反应只需要在初始反应时还原剂为其提供一个电子(图 2A)。为了分析 O_2 和 H_2O_2 作为 LPMOs 共底物的差异，Hangasky 等[56]发现 H_2O_2 依赖的氧化反应是非特异性的，而 O_2 依赖的反应是区域选择性的，但这一说法仍然备受争议。然而，越来越多的研究证明 LPMOs 可以利用 H_2O_2 作为共底物，其反应速率明显高于 O_2[57-58]。例如，Müller 等[58]发现，在 H_2O_2 为底物的反应中，LPMOs 的活性几乎比 O_2 反应高 2 个数量级。同时，研究表明，高浓度的 H_2O_2 可以氧化灭活 LPMOs，其浓度对 LPMOs 活性影响较大[59-60]，而当 LPMOs 与底物结合时，其失活的可能性要低得多[59]。因此，H_2O_2 在 LPMOs 催化作用中起着关键作用，但其具体作用机制还有待进一步研究。

LPMOs 通过氧化裂解糖苷键，根据氧化位点的不同可将其分为 LPMO-1、LPMO-2 和 LPMO-3，它们不同氧化位点的机理引起了研究者们的广泛关注。如图 2B 所示，LPMO-1 仅氧化多糖的 C1 位置，产生内酯产物并转化为醛

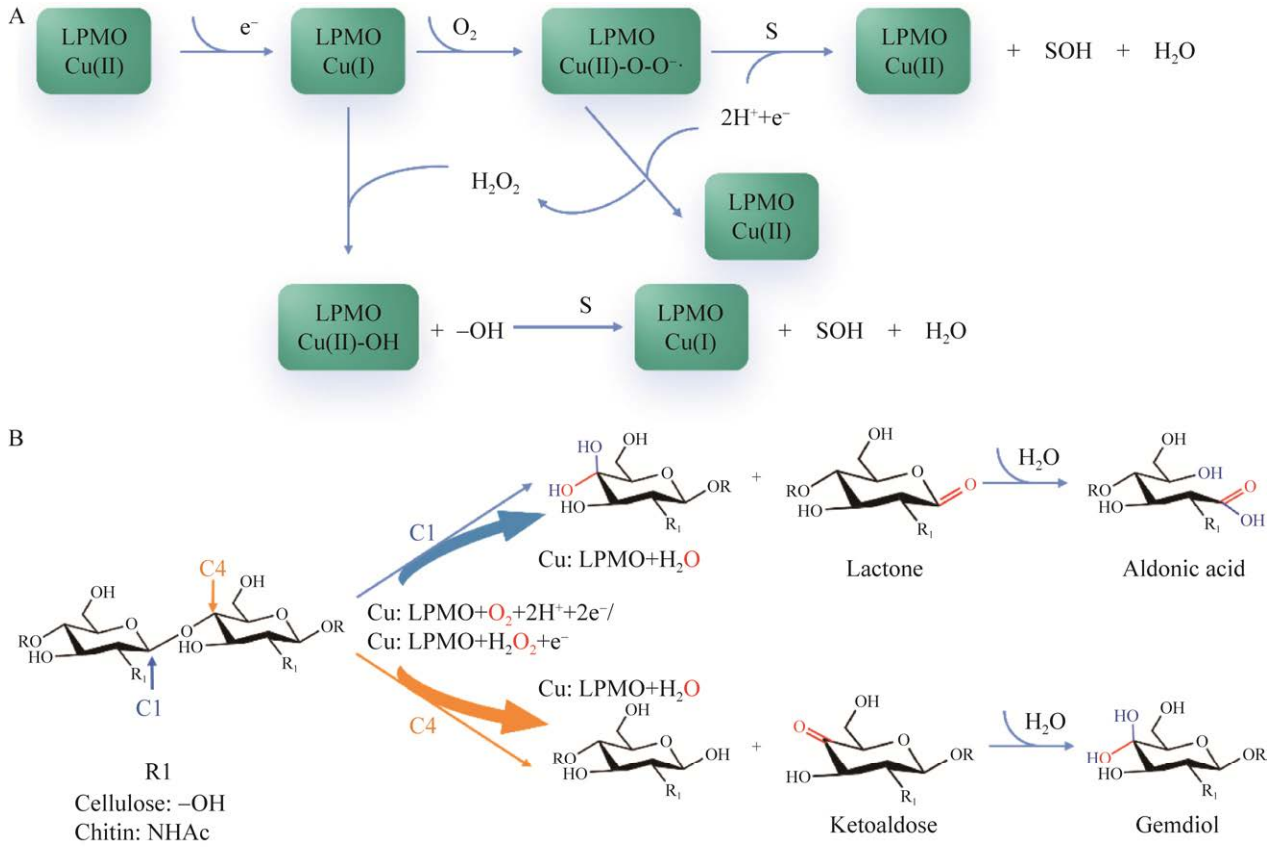

图 2　LPMOs 的催化机制

Figure 2　Catalytic mechanism of LPMOs. A: The reaction schemes for LPMOs. B: Overview of LPMOs with C1 and/or C4 oxidations. S: Polysaccharide substrate.

糖酸。LPMO-2 只氧化多糖的 C4 位置，生成 4-酮醛糖，酮醛糖水合生成偕二醇。LPMO-3 氧化多糖的 C1 和 C4 位置，生成醛糖酸和 4-酮醛糖[23,61]。此外，近期研究还发现部分 LPMOs 可以氧化 C6 位，并不裂解糖苷键[62-63]。结果表明，LPMOs 与底物结合的芳香族氨基酸对其区域选择性影响较大[64]。例如，Danneels 等[65]将来自于红褐肉座菌 LPMO9A 的 Tyr24 和 Tyr211 残基突变为 Ala，发现 Y24A 增强了 C1 氧化，对 C4 氧化变化不大，而 Y211A 增强了 C4 氧化，减弱了 C1 氧化，其他研究报告也证实了类似的结果。此外，Vu 等[15]证实，残基 20 附近约有 12 个氨基酸在 LPMO 的 C4 氧化中发挥重要作用，进一步的突变研究正在进行中。令人惊讶的是，目前报道的 AA10 家族要么有 C1 氧化位点，要么同时有 C1 和 C4 氧化位点，但未单独发现 C4 氧化位点，这可能是由活性位点铜与配体在轴向位置上的可达程度影响的[66]。由于实验数据有限，目前只对其催化机理进行了初步研究，不同 LPMOs 的区域选择性可通过氨基酸突变和缺失来进一步解释。

3　LPMOs 的结构与功能

LPMOs 的来源非常广泛，但其结构却有着高度的保守性，目前，LPMOs 的晶体结构已被解析[以来源于嗜热子囊菌(*Thermoascus aurantiacus*)

的 *Ta*LPMO 为例，PDB ID：3ZUD，1.25 Å]，其核心结构呈 β 三明治，该结构包含 10 个典型的 β 折叠，相邻的 β 折叠之间由 loop 环相连(图 3A)[11]。2011 年，Quinlan 等[11]探究了 *Ta*LPMO 的活性位点，发现该位点由保守的 1 个酪氨酸、2 个组氨酸和 1 个铜离子组成，其中，第 1 个组氨酸被甲基化修饰。铜离子与 2 个组氨酸的侧链和其中 1 个组氨酸的氨基末端的氮原子相连形成一个“T 型”结构，该结构被称为组氨酸支架(图 3B)。与一般酶不同的是，LPMOs 的活性位点呈平面结构，并不位于深的凹槽或隧道内，研究表明，该平面结构的拓扑特征与 LPMOs 的功能密切相关[67-68]。

为得到具有工业应用价值的 LPMO，近年来，对 LPMO 的分子改造越来越引起人们的关注，以期获得高立体选择性、高活性、强稳定性的优良生物催化剂。2019 年，Jensen 等[69]对来源于天蓝色链霉菌(*Streptomyces coelicolor*)的 LPMO 进行结构解析及多序列比对，通过筛选理性设计的突变体库，将具有纤维素催化活性的 *Sc*LPMO10C 转化为具有几丁质活性的 LPMO，表明底物结合平面结构对底物特异性的重要作用。2020 年，Zhu 等[70]对来源于海洋细菌 *Hahella chejuensis* 的 *Hc*LPMO10 进行定向进化，发现位于纤维素结合模块(cellulose binding module, CBM)上的 N526S 位点对纤维素的催化活性具有重要影响。2020 年，Liu 等[71]通过对来源于嗜热毁丝霉(*Myceliophthora thermophile*) *Mt*C1LPMO 的 loop 2 进行定点突变，得到了酶活性增加的突变体 R17L。

4 LPMOs 的活性分析与测定方法

与糖苷水解酶相比，LPMOs 的催化性能并不能被直接测定。首先，催化后只有少量的可溶性醛糖酸/酮醛糖低聚物被释放出来，而大部分氧化产物与不溶底物仍然保持完整；其次，LPMOs 介导的 C1 和/或 C4 氧化，由于离子可能来自同分异构体金属加合物的混合物，因而不容易被质谱识别；最后，LPMOs 的氧化能力与氧化后的不溶性多糖底物成正比，但对不溶性底物的定性和定量分析却一直是难点。近几年，有关 LPMOs 活性分析与检测的方法不断被开发出来，研究者们在传统色谱、质谱和快速检测方法的基础上进行了深入研究和改进，这些方法可适用于许多不同的应用场景，表 2 总

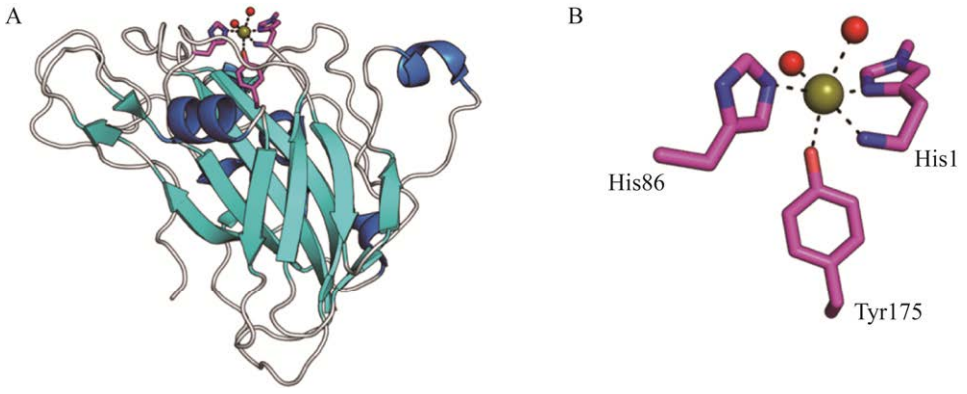

图 3 *Ta*LPMO 三维结构及活性位点示意图[11]

Figure 3 3D structure and active site diagram of *Ta*LPMO[11]. A: 3D structure of *Ta*LPMO. B: “T” histidine scaffolds.

表 2 LPMOs 各种检测方法优缺点的总结

Table 2 Summarized advantages and disadvantages of the LPMOs detection methodologies

Features methods	Qualification or quantification	Targets (soluble/insoluble)	Stability	Accuracy	Ability of activity comparison	Quickness/ Convenience
MALDI-TOF-MS	Qualification	Soluble	High	High	Weak	High
HPAEC-PAD	Both	Soluble	High	High	High	Weak
XPS	Both	Insoluble	High	High	High	Weak
Fluorescent labelling	Quantification[a]	Insoluble	Medium	Medium	Medium	High
Isotope labelling	Quantification[a]	Insoluble	Medium	High	High	Weak
Nickel ions absorption	Quantification[a]	Insoluble	Weak	Medium	High	High
Viscosity changes	Quantification[a]	N.a.[b]	Medium	Medium	Medium	High
Peroxidase activity	Quantification[a]	N.a.[b]	Medium	Medium	High	High
Confocal/AFM	Qualification	Insoluble	High	Weak	Weak	Medium

[a]: A credible method which detect the LPMO's product is necessary prior to this assay; [b]: Not available. This assay not target on detection of the soluble or insoluble oxidation products.

结了 LPMOs 活性测定方法，并对其优缺点进行了系统分析。

可溶性产物的检测比不溶性产物的检测容易。最方便、可靠、快速、有效的方法是基质辅助激光解吸/电离飞行时间质谱法(matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, MALDI-TOF-MS)，该方法灵敏度极高，可以检测微量物质，对 LPMOs 等活性较低的酶的检测效果很好，但缺点也很明显，即只能进行定性分析而不能定量分析；另一方面，由于其灵敏度高，其他酶或污染物很可能会影响检测结果，表现为高本底和杂峰的出现，从而更难获得准确的质谱。此外，高效阴离子色谱(high performance anion exchange chromatography, HPAEC)是目前使用最广泛的 C1 氧化可溶性产物分析方法，具有高灵敏度、高稳定性、高准确性的特点。

上述方法分析的是 LPMOs 反应过程中释放的可溶性寡醛酸产物，而不能分析 LPMOs 对不溶性多糖底物的影响。在实际反应中，不溶性产物的比例受多种因素的影响，如反应体系的设置、同一底物的不同形态(如 Avicel 和 PASC)、底物的浓度等[43,68,72]。X 射线光电子能谱分析(X-ray photoelectron spectroscopy, XPS)技术可用来检测不溶性产物，但 XPS 的使用成本很高，这极大地限制了它的应用。特异性标记和氧化位点检测是检测 LPMOs 在不溶性底物上氧化效率的主要方法，常用的标签可以是荧光基团(fluorescent labelling)、放射性同位素(isotope labelling)或其他容易检测的基团。与荧光或同位素标记类似，一种更简单的方法是基于镍离子吸附法(nickel ions absorption)，通过检测羧酸盐吸附镍离子所引起的游离镍离子浓度的差异来转换 LPMOs 氧化所引起的羧基数量[73]。

有一些方法并不直接针对 LPMOs 反应产物，而是通过间接测量 LPMOs 反应带来的其他参数的变化来检测 LPMOs 的活性。Kojima 等[26]通过观察 LPMOs 引起的底物动态黏度变化(viscosity changes)来分析 LPMOs 的活性，并成功观察到以葡甘露聚糖、羧甲基纤维素、阿拉伯木聚糖和木糖葡聚糖为底物时的黏度下降。这种简单通用的方法的局限性在于只能测定可溶性多糖而不能测定不溶性多糖，特别是纤维素和甲壳素这 2 种最重要的生物质。LPMOs 在

O_2存在时被还原生成 H_2O_2，溶液中的 H_2O_2可被检测到，因此，研究者们成功开发了基于LPMOs 过氧化物酶活性(peroxidase activity)的测定方法。

除了上述直接或间接的分析方法外，通过成像方法观察 LPMOs 催化过程中底物的微观形态变化也可以作为定性分析的手段。研究表明，经过 LPMOs 处理和荧光染色后，纤维素底物在激光共聚焦显微镜下表现出透射和荧光，而对照组则无这种现象[74]。该方法适用于LPMOs 活性的定性分析。同时，观察荧光信号在衬底上的位置有助于确定 LPMOs 的选择性。此外，原子力显微镜(atomic force microscopy, AFM)也可用于观察 LPMOs 氧化引起的基质细微变化。

5 LPMOs 的异源表达与调控

LPMOs 来源广泛，但利用自身来源菌株发酵获得的 LPMOs 在产量和分离方面存在限制。为了提高 LPMOs 的生产效率，推进其工业应用，在其他宿主中进行异源表达是一种有效的手段。目前，LPMOs 主要的表达宿主是大肠杆菌、芽孢杆菌、毕赤酵母(*Pichia pastoris*)和一些真菌菌株。由于 AA9 在起源上都是真菌[74]，大部分 AA9 在常见的真菌表达宿主中都有异源表达，而在细菌中表达较少。作为最常用的真核表达系统，毕赤酵母因其基因操作技术成熟，已广泛用于 AA9 的异源表达[22,75]。考虑到 AA9 的第 1 个氨基酸是活性位点，在构建质粒时，AA9 成熟肽的 N 端可以直接连接到信号肽识别位点，这样，信号肽酶就可以正确地裂解得到具有正确 N 端序列的 AA9[26,76]。另一种方法是在质粒的构建过程中加入合适的酶切割位点，用相应的酶在表达后对蛋白进行切割，确保第 1 个残基是 His[77]。除了毕赤酵母，一些 AA9 也已经在其他真菌表达宿主中重组表达，如 *Aspergillus oryzae*、*M. thermophila*、*T. reesei*、*A. nidulans*、*Penicillium verruculosum*[29-30,42,44,78]，但可能存在转化和操作困难等问题。在这些真菌表达宿主中表达的 AA9 的 N 端残基 His 被甲基化[11,60,79]，这使得 AA9 更能抵抗 H_2O_2引起的氧化失活[60]。因此，甲基化修饰 N 端组氨酸可以提高 AA9 的稳定性，对其工业应用具有重要意义。

为了提高 AA9 的生产效率，在真菌表达宿主中，已经有多种信号肽用于分泌表达 AA9。当以毕赤酵母为宿主时，α-因子信号肽[76-77,80]和 AA9 的原生信号肽[75,81]是最常用的信号肽。例如，Ladevèze 等[82]在毕赤酵母中使用了原生信号肽和 α 因子信号肽表达 3 种蛋白，研究发现，*Gc*LPMO9A 和 *Gc*LPMO9B 利用其原生信号肽获得的蛋白产量要高于 α-因子信号肽，而 *Gc*LPMO9C 使用 α-因子信号肽获得更高的蛋白产量。然而，使用原生信号肽的 3 种酶的比活性都高于 α 因子信号肽，这是因为使用原生信号肽分泌的 LPMOs 具有更多正确的 N 端 His[82]。Kadowaki 等[83]在米曲菌中使用 2 种信号肽(原生信号肽和 pEXPYR 载体上的糖淀粉酶信号肽)表达 *Mt*LPMO9J，发现只有原生信号肽能够成功分泌具有正确 N 端 His 的 *Mt*LPMO9J。综上所述，当 AA9 在真菌中分泌表达时，与其他信号肽相比，使用其自身信号肽分泌的 N 端正确重组蛋白会更多。然而，在真菌表达宿主中优化 AA9 信号肽的研究并不多，因此，可以利用不同的信号肽进一步提高 AA9 的生产效率，信号肽与 LPMOs 的构象关系也非常值得研究。

除了上述真菌表达宿主外，LPMOs 也在大肠杆菌和芽孢杆菌中进行了表达尝试，但在大肠杆菌中表达时，大多出现胞内表达。例如 de

Gouvêa 等[84]在大肠杆菌中异源表达 *Aspergillus fumigatus* 衍生的 *Af*AA9B 基因，结果发现 *Af*AA9B 形成了包涵体。用载体 pET-21a 去除其原有信号肽后，实现了该基因在大肠杆菌中的胞内可溶性表达[85-86]。此外，Guo 等[87]在大肠杆菌和解淀粉芽孢杆菌中成功分泌 *Mt*C1LPMO，发现不同的信号肽对其分泌水平有不同的影响。然而，在大肠杆菌和解淀粉杆菌中表达的 *Mt*C1LPMO 的比活性低于毕赤酵母，这可能是由于毕赤酵母对该蛋白进行了糖基化[88]，但当前文献中缺乏糖基化对 LPMOs 活性影响的系统研究[89]。

AA10 大多来源于细菌，因此 AA10 主要在芽孢杆菌和大肠杆菌中异源表达[90]。当 AA10 在大肠杆菌中表达时，大部分 AA10 可以通过信号肽到达周质空间[34,37,91]，然后正确断开，保留蛋白质 N 端的 His 残基。一些 AA10 使用其原生信号肽表达[34]，一些使用来自其他蛋白质的信号肽[92]，不同的信号肽对 AA10 的表达效率影响不同。Courtade 等[92]比较了不同 AA10 的信号肽，发现 *Sm*AA10A (CBP21)的信号肽优于其他 5 种蛋白的信号肽。同时，信号肽 *Sm*AA10A 可以实现多种蛋白的高效表达[93]。然而，通过比较 12 种不同的信号肽和 *Sm*AA10A 的原生信号肽，Yang 等[91]发现 pelB 是提高 *Sm*AA10A 产量的最有效信号肽。许多不同来源的 AA10 也可以用 pelB 成功表达[37,41]。据文献报道 AA10 在无信号肽时可以在大肠杆菌中成功表达[94-96]，并通过避免分泌到周质空间来提高产量[96]。要获得有活性的 AA10，需要在其第 1 个 His 残基前面插入一个酶裂解位点。比如 Gregory 等[96]使用 pET-SUMO 载体表达 *Ba*AA10 (来源于 *Bacillus amyloliquefaciens*)，将成熟肽置于载体 SUMO 蛋白酶裂解位点后面。

除了大肠杆菌外，一些 AA10 也被克隆并在芽孢杆菌宿主中表达[97]。当在芽孢杆菌表达时，蛋白质可直接被分泌到胞外培养基中，进而可减少下游分离和纯化的成本[98]。相比于大肠杆菌复杂的周质分离，利用芽孢杆菌表达蛋白的纯化过程更为简单。例如，Yu 等[97]使用枯草芽孢杆菌表达 *Bat*LPMO10 时，蛋白产量比大肠杆菌高 3.7 倍。然而，与大肠杆菌相比，芽孢杆菌中的质粒稳定性较差，质粒转化和原生质体制备困难，实验操作性较差[99]。与在毕赤酵母中不同，当使用非原生信号肽时，LPMO 在大肠杆菌或芽孢杆菌中的表达更有效[87,91-92,100]。此外，还有个别的 AA10 在其他宿主中异源表达，如 *Thermobifida fusca* 中的 *Tf*AA10A 可以在 *Synechococcus elongatus* 中进行表达[101]。

除 AA9 和 AA10 外，目前鉴定的 AA11 和 AA13–AA17 数量较少，它们在不同宿主中也存在异源表达。从源菌株中克隆的 AA11[42-43]和 AA17[53]，也实现了在大肠杆菌中的表达。AA13 部分在源菌株中表达[44]，还有一些克隆并在其他真菌中表达[45-46]。AA14 和 AA16 分别利用其原生信号肽在毕赤酵母中成功表达[49,52]。此外，AA15 也成功实现在大肠杆菌和毕赤酵母中的异源表达[50-51]。

综上所述，总结 LPMOs 各家族的异源表达，主要表达宿主为大肠杆菌和毕赤酵母。不难发现，从真菌中获得的 AA9 更适合在毕赤酵母中表达，产率也高于大肠杆菌。相比之下，AA10 主要来源于细菌，在大肠杆菌中的表达更为成功。AA11、AA13、AA14、AA16 和 AA17 都来自真菌，尚未发现相对较优的首选表达宿主。目前对 AA11 和 AA17 的研究数据表明，它们只在大肠杆菌中进行过异源表达，可能是由于对它们的研究较少的缘故。因此，随着越来越多 LPMOs 的发现，LPMOs 对表达宿主的偏好性是非常值得系统研究的领域。

6 LPMOs 的应用研究

6.1 LPMOs 在生物能源方面的应用

木质纤维素类生物质能作为可再生碳源，是仅次于煤炭、石油、天然气的第四大能源，它的开发利用是应对全球气候变化、能源短缺和环境污染最有潜力的解决方案之一。木质纤维素具有“生物质抗降解屏障”的特征[102]，因此，经济高效的木质纤维素降解酶系统开发是解决问题的关键，这也是制备纤维素乙醇等生物能源的基础。由于木质纤维素组分与结构的异质性和多样性，其充分降解依赖多种纤维素酶与辅酶的协同作用，然而，受限于传统水解酶降解复杂结晶多糖的低效率，这些生物质资源还不能被有效利用。2010 年，LPMOs 的发现为突破木质纤维素糖化瓶颈带来重大机遇。LPMOs 的开发与应用，将为降低农林废弃物生物质转化的用酶成本、促进低碳绿色纤维素高值化利用做出重要贡献。

6.2 LPMOs 在纳米纤维方面的应用

纳米纤维素在自然界中的含量非常丰富，是一种极具发展前景的纳米材料。纳米纤维素具有许多优异的特性，如出色的机械性能、可调节的表面化学性质、低毒性、可生物降解性和生物相容性等。纳米纤维素可应用于药物输送、伤口敷料、组织工程支架等[103]，在生物医学领域应用前景广阔。

近年来的研究发现，LPMOs 可削弱纤维内聚力，促进纤维断裂，同时保持纤维素的结晶度，可用于处理纤维素纤维，以制备纳米级纤维素。相比于传统的纳米纤维制备方法，该过程低耗能、低毒性，具有制备纤维素纳米材料的绿色潜力[104]。

6.3 LPMOs 在植物防御方面的应用

LPMOs 是存在于真菌、细菌和病毒中的铜依赖性酶，对植物的感染以及降解植物体中纤维素发挥至关重要的作用。自从发现以来，LPMOs 在生物质转化中的研究已经取得了重大进展。最近在真菌和卵菌纲(AA16)以及昆虫(AA15)中发现了其他 LPMOs 家族，表明 LPMOs 可能还参与了其他生物过程，例如克服植物防御。美国俄克拉荷马州立大学 Andrew J. Mort 教授在权威期刊 Trends in Plant Science 发表了综述论文，Do lytic polysaccharide monooxygenases aid in plant pathogenesis and herbivory?从植物防御的角度全面概述不同的 LPMOs 家族的潜在作用，以及它们在制定新的策略以实现对植物病原菌和虫害的保护方面的多重意义[54]。

7 展望

LPMOs 通过氧化裂解破坏纤维素的结晶结构，使其结构松散，为糖苷水解酶提供更多的结合位点，对纤维素的高效酶解起到重要作用，是极具开发潜力的纤维素降解酶。NOVOZYME 是世界上最好的酶制造商之一，最近升级了其商业纤维素降解酶，其中就包括 LPMOs[4]。然而，LPMOs 从实验室到大规模生物炼制产业的可扩展应用仍然存在诸多问题和关键挑战。例如，LPMOs 的催化机制尚不明确，重组 LPMOs 的产量还不适用于实际的生物炼制工艺，目前高活力和强稳定性兼备的 LPMOs 仍然稀缺等等，这都成为 LPMOs 在生物质转化领域进行工业应用的重要瓶颈。因此，未来的研究应该着力于解决这些关键问题和挑战，以促进木质纤维素在维持全球碳循环和生物炼制产业发展中的高值化和资源化应用。

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