微生物学报
Acta Microbiologica Sinica
2020, 60(12): 2635–2649
http://journals.im.ac.cn/actamicrocn
DOI: 10.13343/j.cnki.wsxb.20190504

白蚁消化系统转化和降解木质纤维素酶研究进展

蒋宇彤，张硕，林子佳，倪金凤*

山东大学微生物技术研究院，微生物技术国家重点实验室，山东 青岛 266237

摘要：木质纤维素是地球上最丰富的有机聚合物，白蚁是古老但进化最成功的高效木质纤维素降解者之一。了解白蚁降解高度抗性植物聚合物的机制对工业上生物质能源转化和生物仿生设计有重要的借鉴和指导价值。白蚁和其共生微生物产生的木质纤维素酶在其转化利用木质纤维素上发挥着重要作用。本文从来源作用方面对白蚁自身及其肠道原虫、细菌和真菌产生的纤维素酶、木聚糖酶和漆酶等酶研究概况进行了总结，对其存在的问题和前景进行了展望。本综述有助于全面了解白蚁消化系统木质纤维素酶的基因种类、来源、分布、表达以及酶活性和功能。

关键词：白蚁，木质纤维素降解，纤维素酶，木聚糖酶，漆酶

木质纤维素是地球上由植物产生的最丰富的有机聚合物，它不溶于水且对酶水解具有高度抗性，一物降一物，自然界进化出一些能够高效降解木质纤维素的体系，白蚁是其中最成功的动物群体之一。一方面，白蚁一年能消耗 30–70 亿 t 的木质纤维素[1]，具有高效的食木性[2]；另一方面，白蚁作为最古老的社会性昆虫之一，在地球生态系统尤其是热带雨林有机物质循环中发挥着重要作用，是热带森林和沙漠生态系统中最重要的分解者[3–4]。与降解木质纤维素的其他生物相比，白蚁有较高的转化率，白蚁与其共生微生物的协同作用可以消化 74%–99%的纤维素和 65%–87%的半纤维素[5]。食木白蚁在 24 h 内可以有效降解利用多种植物基质，培菌白蚁在几小时内就可以完成木质素的降解[6–7]。白蚁取食后，无需对食物进行高温、高压和强碱等特殊处理，通过咀嚼和其内外共生微生物的协同作用，就能够将食物转化成生存所需的营养和能量。与工业炼制过程相比，白蚁木质纤维素转化是一个高效而温和的系统，故食木白蚁肠道有“世界上最小的生物反应器”之称[8]。了解白蚁降解木质纤维素的体系和机制，不仅能为生物质的转化利用提供新的思路、为生物仿生设计提供依据和新的见解，也能为工业应用提供重要的菌群和酶资源。

基金项目：国家自然科学基金(31970119，31272370)

***通信作者**。Tel/Fax：+86-532-58631564；E-mail：jinfgni@sdu.edu.cn

收稿日期：2019-10-30；**修回日期：**2020-01-08；**网络出版日期：**2020-11-09

在长期的进化中，白蚁与消化系统内和菌圃外的不同微生物类群形成了多样化的共生体系，其中共生微生物发挥重要作用，这方面已经有很多系统且全面的文章介绍[3,9–12]。鉴于此，本文将主要针对白蚁和其共生微生物产生的木质纤维素相关降解酶研究进展进行介绍。

1 白蚁概述

白蚁属于昆虫纲蜚蠊目，与蟑螂有非常近的进化关系[13]。白蚁大约有 3000 种，根据肠道中是否存在原生动物，可将白蚁分为低等白蚁和高等白蚁两类[14]。低等白蚁涵盖 6 个科[15]，包括澳白蚁科(*Mastotermitidae*)、草白蚁科(*Hodotermitidae*)、原白蚁科(*Termopsidae*)、木白蚁科(*Kalotermitidae*)、齿白蚁科(*Serritermitidae*)和鼻白蚁科(*Rhinotermitidae*)。高等白蚁仅有白蚁科(*Termitidae*)，但数目却占所有已知白蚁种类的 75%。白蚁科可分为 4 个亚科：大白蚁亚科(*Macrotermitinae*)、象白蚁亚科(*Nasutitermitinae*)、尖白蚁亚科(*Apicotermitinae*)和白蚁亚科(*Termitinae*)，其中大白蚁亚科的白蚁与特定真菌——鸡枞菌(*Termitomyces*)共生，在白蚁巢内建立菌圃，培养小白球菌为白蚁提供营养[16–17]，所以大白蚁亚科的白蚁又称为培菌白蚁(Fungus-growing termite)。培菌白蚁主要分布在非洲和亚洲热带地区，在植物废弃物的降解过程中发挥重要作用[18]。白蚁是社会性昆虫，所有个体都生活在群体之中，不能单独存活。一个白蚁群体通常由繁殖蚁(蚁王和蚁后)、工蚁、兵蚁、幼蚁和卵等组成，它们与其生活的巢体构成一个超级有机体。

白蚁消化系统一般由唾液腺(salivary glands，SG)、前肠(foregut，FG)、中肠(midgut，MG)和后肠(hindgut，HG)组成。与低等白蚁肠道系统相比，高等白蚁肠道更为复杂。以高山象白蚁(*Nasutitermes takasagoensis*)为例，其中肠向后肠延伸，形成了包含不同 P 区(P1–P5)的混合区[19]。白蚁后肠含有丰富的微生物群，一般包括细菌、真菌和古菌，低等白蚁后肠还包括原生生物。

2 木质纤维素及其降解酶

木质纤维素是植物细胞壁的主要组成成分，是地球上最丰富的可再生生物质，占据了地球 90%以上光合作用产生的生物质资源。天然的木质纤维素材料由纤维素、半纤维素和木质素组成。其中，纤维素是木质纤维素中最简单的成分，由许多 D-吡喃葡萄糖经过 β-1,4-糖苷键连接而成，约占木质纤维素含量的 28%–50%。纤维素水解成为葡萄糖需要以下 3 种主要酶参与：1) 内切 β-1,4-葡聚糖酶(endo-glucanase，EG，EC 3.2.1.4)；2) 外切葡聚糖酶(exo-glucanase 或 Cellobiohydrolases，CBH，EC 3.2.1.91)；3) β-葡萄糖苷酶(β-glucosidase，BG，EC 3.2.1.21)。EG 酶作用于纤维素的半结晶区/无定型区，随机切割疏松纤维素分子内部的糖苷键；CBH 从纤维素分子的还原末端或者非还原性末端进行切割，产生纤维二糖或者葡萄糖；BG 酶负责水解小于六个葡萄糖单元组成的寡糖类[5,20]。

半纤维素在木质纤维素中的含量为 20%–30%，在自然界中的含量仅次于纤维素。半纤维素是由多种不同类型的单糖构成的异质多聚体，包括木聚糖、甘露聚糖、半乳聚糖和阿拉伯聚糖等，其中木聚糖在半纤维素中含量最丰富[21]。木聚糖的完全水解需要内切 β-1,4-木聚糖酶(endo-β-1,4-xylanase，EC 3.2.1.8)和 β-木糖苷酶

(β-xylosidase，EC 3.2.1.37)共同作用完成[22]。此外，半纤维素的降解还需要其他一些辅助酶的作用，如乙酰木聚糖酯酶、α-葡萄糖醛酸糖苷酶、α-L-阿拉伯呋喃糖苷酶和乙酰酯酶等。

木质素在木质纤维素中的含量为18%–30%，是由苯丙烷单元(愈创木基丙烷、紫丁香基丙烷和对羟苯基丙烷)通过醚键和C-C键相连接形成的多聚体，木质素与多糖紧密结合并与半纤维素共价结合形成围绕纤维素微纤维的基质，共同形成木质纤维素。在植物体中，木质素包裹在纤维素的外面，功能之一是保护植物不受外界微生物的侵蚀。这种保护作用阻碍了纤维素酶与纤维素的接触，成为影响纤维素降解的重要因素[23]。木质素的降解主要依赖三种酶：木质素过氧化物酶(Lignin peroxidases，Lip，EC 1.11.1.14)、锰过氧化物酶(Mn peroxidases，MnPs，EC 1.11.1.13)和漆酶(Laccases，Lac，EC 1.10.3.2)。

3 白蚁和其共生微生物由来木质纤维素降解酶

传统观点认为白蚁自身不产生纤维素酶，主要依赖与其共生的微生物消化降解木质纤维素。后来，随着白蚁内源性纤维素酶的发现和基因克隆，改变了人们以往的看法和观点。研究表明，白蚁能够依赖自身及其体内外共生微生物产生酶的分工协作高效降解木质纤维素，这些木质纤维素降解酶包括：(1) 白蚁自身产生的酶；(2) 体内肠道微生物产生的酶；(3) 体内外真菌产生的酶。

3.1 白蚁自身产生的木质纤维素酶

自Watanabe等于1998年在《自然》杂志首次报道白蚁中存在内源性纤维素酶基因以来[24]，现已从多种白蚁如散白蚁、台湾乳白蚁、恒春新白蚁、食木象白蚁和培菌白蚁中克隆了许多内源性的纤维素酶基因[20,25–27](表1)。如前所述，纤维素酶包括EG、CBH和BG三种酶，但目前研究还没有发现白蚁中存在内源性CBH，所以早期文献及本文所述的白蚁内源性纤维素酶主要指EG和BG两种酶。不同白蚁来源的纤维素酶基因具有高度同源性，EG都属于糖苷水解酶家族9 (GH9)，而BG属于糖苷水解酶家族1 (GH1)。反转录PCR实验证实在低等白蚁中，EG和BG两种酶的分泌位点都在唾液腺，这与低等白蚁唾液腺具有高酶活性的结论一致。而在高等白蚁中，EG由中肠分泌产生，BG由唾液腺和中肠两个部位产生。来自于不同白蚁(不管是低等白蚁还是高等白蚁)的EG和BG两种酶，分子量大小相似，如EG约为47 kDa，BG约为55 kDa，氨基酸序列同源性为70%–80%，并且EG酶只有一个催化结合域，没有纤维素结合域。

为了深入了解白蚁纤维素酶的功能及其在生物质资源转化方面的潜在应用前景，目前已经实现了EG和BG两种酶基因在不同系统包括大肠杆菌、酵母、曲霉和昆虫细胞的异源表达[20,28]，采用酶分子定向进化技术，获得了高比活性且高耐热性的白蚁内切葡聚糖酶[29]。栖北散白蚁和高山象白蚁的内切葡聚糖酶基因通过优化实现了在曲霉中的大量表达和纯化，动力学研究表明曲霉中异源表达的重组酶比活性有明显提高[30]，在毕赤酵母中表达的EG酶突变体在pH 4–11范围内仍能保持高稳定性[28]。

结合白蚁肠道纤维素酶活性分布，研究表明，食物经白蚁咀嚼变成小的木质颗粒，进入消化道后与唾液腺和中肠分泌的GH9和GH1家族纤维

表 1. 白蚁来源木质纤维素降解酶

Table 1. Termite-derived lignocellulose degrading enzymes

Enzyme type	Enzyme names	Termite species	GH family	References
Endo-β-1,4-glucanase	RsEG	*Reticulitermes speratus*	GH9	[24]
Endo-β-1,4-glucanase	MbEG1	*Macrotermes barneyi*	GH9	[25]
Endoglucanase	RsEGm	*Reticulitermes speratus*	GH9	[29]
Endo-β-1,4-glucanase	A18, PA68	*Reticulitermes speratus* *Nasutitermes takasagoensis*	GH9	[30]
Endo-β-1,4-glucanase	RsEG, NtEG	*Reticulitermes speratus* *Nasutitermes takasagoensis*	GH9	[31]
Endo-β-1,4-glucanase	YEG1, YEG2	*Reticulitermes speratus*	GH9	[36]
Endoglucanase	NtEgl	*Nasutitermes takasagoensis*	GH9	[37]
Endoglucanase	nCfEG, tCfEG	*Coptotermes formosanus*	GH9	[38]
Endo-β-1,4-glucanase	G91A,Y97W, K429A	*Reticulitermes speratus*	GH9	[39]
Endo-β-1,4-glucanase	BEVS-expressed enzyme	*Reticulitermes flavipes*	GH9	[100]
β-glucosidase	MaBG	*Microcerotermes annandalei*	GH1	[26]
Glucosidase	RsBGI,RsBGII	*Reticulitermes speratus*	GH1	[27]
Glucosidase	ND	*Neotermes koshunensis*	GH1	[40]
Glucosidase	ND	*Reticulitermes flaviceps*	GH1	[41]
Glucosidase	rfbgluc-1	*Reticulitermes flavipes*	GH1	[42]
Glucosidase	BglB	*Reticulitermes santonensis*	GH1	[43]
Glucosidase	G1mgNtBG1	*Nasutitermes takasagoensis*	GH1	[44]
Glucosidase	G1NkBG	*Neotermes koshunensis*	GH1	[45]
Glucosidase	NkBgl	*Neotermes koshunensis*	GH1	[46]
β-glucosidase	MbmgBG1	*Macrotermes barneyi*	GH1	[47]
Glucosidase	bgl5, bgl7, bgl9	*Macrotermes annandalei*	GH1	[48]
Glucosidase	ND	*Coptotermes formosanus*	GH1	[50]
Glucosidase	bgl-gs1	*Globitermes brachycerastes*	GH1	[75]
Laccase	RfLacA, RfLacB	*Reticulitermes flavipes*	ND	[33]
Laccase	Lac1	*Coptotermes formosanus*	ND	[35]

ND: not determined.

素酶混合，EG 作用于木质颗粒的疏松区域，将其初步降解成低聚寡糖和纤维二糖，而 BG 进一步将纤维二糖水解成葡萄糖为白蚁所利用。用 C^{13} 同位素标记的食物饲喂湿木白蚁 *Hodotermopsis sjostedti*，结果表明白蚁内源纤维素酶在纤维素消化过程中起主要作用[1]，利用 RNA 干扰技术针对台湾乳白蚁内源的 EG 和 BG 酶基因，使得相应酶活性降低，可导致白蚁体重减轻和死亡率增加，说明白蚁内源纤维素酶在白蚁生长中发挥重要作用[31–32]。

关于半纤维素降解酶，据目前所知，还没有发现白蚁自身由来的木聚糖酶。

转录组学分析发现低等白蚁(黄胸散白蚁)体内含有内源性漆酶(RfLacA 和 RfLacB)，其产生位点在唾液腺并分泌到前肠。通过昆虫杆状病毒表达系统，成功实现了内源性漆酶基因的功能性表达和纯化。重组漆酶对木质素单体——芥子酸和 4 种苯酚底物有较强的活性，而对 4 种黑色素前体几乎没有活性，另外观察到重组漆酶可以修饰碱性木质素，这些研究表明了白蚁内源性漆酶可能在木质素降解过程中发挥作用[33–34]。此外，在台湾乳白蚁(*Coptotermes formosanus*)中也发现了内

源性漆酶基因 *lac1*，其在昆虫 Sf9 细胞中实现了异源表达纯化，纯化的漆酶在 pH 4.5–7.5 有活性，对氢醌底物有较高的作用活性(305 mU/mg)，研究发现 4.85 mmol/L 以上浓度的 H_2O_2 可显著抑制漆酶 Lac1 的活性($P<0.01$)。漆酶 Lac1 同黄胸散白蚁漆酶 RflacA 和 RflacB 一样，主要在唾液腺和前肠中表达，在中肠或后肠中很少表达(表 1)。这 3 种漆酶都属于酚氧化漆酶，不同之处在于漆酶 Lac1 在酚氧化反应中活力最高，并且氧化反应过程不需要 H_2O_2 的协助，推测白蚁漆酶 Lac1 可能直接氧化低氧化态势的底物，而木质素中的高氧化态势基团可能被白蚁中的其他酶或通过芬顿反应氧化[35]。目前，在高等白蚁中尚未有漆酶的相关报道，但是本实验室通过对培菌白蚁(黄翅大白蚁)转录组进行分析，发现了多个潜在的基因序列，目前已经克隆获得其全长基因，通过构建及优化其异源表达体系，本实验室将进一步研究培菌白蚁潜在内源性漆酶功能及催化特性。

3.2 白蚁肠道微生物产生的酶

3.2.1 来自低等白蚁共生微生物的木质纤维素降解酶：低等白蚁肠道有原生动物、细菌和古菌 3 种共生微生物。2002 年日本学者通过研究台湾乳白蚁提出了共生微生物产生酶与白蚁自身消化酶协同高效降解木质纤维素的二维学说，并指出在这一协同作用中，原生动物酶系发挥的降解作用可能大于白蚁自身酶系[65]。相比于白蚁的两种内源性纤维素酶，后肠共生微生物编码的纤维素酶更多样。通过肠道微生物分离、构建后肠环境 DNA 文库、宏基因组等高通量测序分析，发现后肠微生物编码 GH3、5、7、8、10、11、26、43、45 和 62 等更多家族的纤维素酶和半纤维素酶基因序列[49–53]。来源于白蚁后肠的原虫或细菌属于 GH5、7、8、45 家族的多个 EG 酶基因和 CBH 酶基因，以及属于 GH1 家族的 BG 酶基因已经被克隆或表达鉴定。低等白蚁后肠细菌主要贡献是将纤维二糖或纤维低聚糖磷酸解。低等白蚁原虫由来的纤维素酶普遍比细菌由来的酶活性高，这暗示白蚁原虫来源的纤维素酶在其纤维素降解中发挥更大作用，而肠道共生菌来源酶呈现低的酶活[54]。应用单细胞宏基因组研究方法表明低等白蚁后肠原生生物可能不参与纤维素消化，发挥作用的是原虫表面的细菌群落，这些细菌群落分泌一系列复杂的糖苷水解酶，将纤维素降解为单体来满足原生生物的代谢需求[55]。

从黄肢散白蚁(*Reticulitermes flavipes*)的共生原虫克隆获得 3 个 GH7 家族的酶基因(*GHF7-3*，*GHF7-5*，*GHF7-6*)，并在昆虫病毒体系中实现了 3 个重组酶的表达，其中 GHF7-3 活性最高，并具有 CBH、EG 和 BG 的多种酶活性(表 2)。在木质纤维素水解过程中，重组酶 GHF7-3 与白蚁内源性酶协同作用，能够增加葡萄糖的产生量[56]。对台湾乳白蚁后肠原生动物 *Pseudotrichonympha grassii* 由来的 GH7 家族 *PgCBH* 基因进行 RNA 干扰实验，降低 *PgCBH* 基因表达水平，CBH 酶活性显著降低，导致了原虫死亡，这表明 *PgCBH* 基因在原虫木质纤维素分解过程中起作用[57]。

目前，已报道进行详细分析鉴定的木聚糖酶主要属于 GH10 和 GH11 家族[20]。2009 年，Arakawa 等首次报道从台湾乳白蚁后肠纯化到 3 种分子量为 17–19 kDa 的 GH11 家族木聚糖酶，RT-PCR 实验证实了该木聚糖酶由后肠的共生鞭毛虫产生[58]，之后有文章报道从桑特散白蚁(*Reticulitermes santonensis*)肠道细菌也分离鉴定到一个 GH11 家族的木聚糖酶[59]。对台湾乳白蚁后肠原生动物进

行宏转录组测序分析鉴定，并在毕赤酵母中重组表达了一个 GH10 家族的木聚糖酶基因[53]，重组表达的木聚糖酶(xyl726)分子量约为 34 kDa，酶比活性为 80 U/mg (表 2)。

3.2.2 来自高等白蚁共生微生物的木质纤维素降解酶：高等白蚁肠道没有共生的原生动物，后肠细菌在高等白蚁木质纤维素降解过程中发挥重要作用。高等象白蚁(*Nasutitermes*)的宏基因组学研究发现后肠含有 700 多个糖苷水解酶基因序列，分别属于 45 个碳水化合物活性酶家族，其中 100 多个基因和纤维素水解相关，包括 GH5 纤维素酶、GH94 纤维二糖/纤维糊精磷酸化酶类和 GH51 内切葡聚糖酶/阿拉伯糖酶等[68]。通过构建 Fosmid 文库和功能筛选，从短角球白蚁(*Globitermes brachycerastes*) 和土垅大白蚁 (*Macrotermes annandalei*) 两种高等白蚁后肠微生物中发现上百个具有不同活性的纤维素酶和半纤维素酶克隆[10,69]。从 50000 个克隆中筛选得到 464 个具有植物多糖降解活性的克隆，其中包括 267 个内切葡聚糖酶、24 个外切葡聚糖酶、72 个 β-葡萄糖苷酶和 101 个内切木聚糖酶等阳性克隆，鉴定了 219 个可能降解纤维素、半纤维素和果胶的碳水化合物水解酶基因[10]。对高等白蚁 *Trinervitermes trinervoides* 后肠细菌进行宏基因组分析，发现 25 种纤维素酶和半纤维素酶的开放阅读框，它们属于 GH5 等 11 个不同家族。其中 8 个基因表达鉴定为内切纤维素酶(GH5C、GH5E、GH5F 和 GH5G)、外切纤维素酶(GH5D)、内切木聚糖酶(GH5H 和 GH11)和 α-岩藻糖苷酶(GH29)。其中 GH11 家族的 Xyl1 是多亚基 β-1,4-木聚糖酶，由催化结构域和 2 个碳水化合物结合域(CBM)组成。CBM 选择性地结合不溶性木聚糖，能够提高水解速率。该木聚糖内切酶在 pH 6 和 50℃条件下活性最佳，能够降解木聚糖产生木二糖和木三糖(表 3)。此外，发现 Xyl1 对天然底物(例如小麦阿拉伯木聚糖)也具有催化能力，这使其成为有效分解复杂植物生物质结构的潜在生物催化剂[70]。这些研究表明白蚁后肠蕴藏了丰富的木质纤维素酶基因，尽管如此，后肠相关的木质纤维素酶活性不高，比如高山象白蚁后肠内切葡聚糖酶的活性远远低

表 2. 原虫来源木质纤维素降解酶

Table 2. Protist-derived lignocellulose degrading enzymes

Enzyme type	Enzyme names	Termite species	GH family	References
Endo-β-1,4-xylanase	xyl726	*Coptotermes formosanus*	GH10	[53]
Xylanase	CfXyn-1,-2,-3	*Coptotermes formosanus*	GH11	[58]
Endo-β-1,4-glucanase	ND	*Coptotermes lacteus*	GH7	[60]
Endoglucanases	ND	*Mastotermes darwiniensis*	GH45	[61]
Endo-β-1,4-glucanase	CFP-eg1	*Coptotermes formosanus*	GH5	[62]
Endoglucanase	RsSymEG1	*Reticulitermes speratus*	GH7	[63]
Endo-β-1,4-glucanase	RsSymEG2	*Reticulitermes speratus*	GH45	[64]
Cellobiohydrolases	ND	*Reticulitermes flavipes*	GH7	[56]
Cellobiohydrolases	PgCBH	*Coptotermes formosanus*	GH7	[57]
Cellulase	ND	*Coptotermes formosanus*	GH7	[65]
Cellulase	ND	*Reticulitermes speratus*	GH45	[66]
Cellulases	Cell-2,-3,-4	*Reticulitermes flavipes*	GH7	[67]

ND: not determined.

表 3. 细菌、真菌来源木质纤维素降解酶

Table 3. Bacteria and fungi derived lignocellulose degrading enzymes

Origin	Enzyme type	Enzyme names	Termite species	GH family	References
Bacteria	Endo-β-1,4-xylanase	mXyl8B8	*Reticulitermes santonensis*	GH11	[59]
	Endo-β-1,4-xylanase	Xyl1	*Trinervitermes trinervoides*	GH11	[70]
	Xylanase	XYL7	*Globitermes brachycerastes*	GH11	[76]
	Xylanase	Xyl-ORF19	*Globitermes brachycerastes*	GH10	[77]
	Xylanase	NtSymX11	*Nasutitermes takasagoensi*s	GH11	[78]
	Xylanase	IIPSP3 xylanase	Wood-feeding higher termites	ND	[94]
	Endo-β-1,4-glucanase	EglC22b	*Reticulitermes labralis*	GH8	[92]
	Endo-β-1,4-glucanase	β-1,4-glucanase	*Anacanthotermes*	ND	[93]
	Laccase	ND	*Cryptotermes brevis*	ND	[95]
Yeast	Xylanase	SSA-1542T	*Reticulitermes chinensis*	ND	[91]
	Xylanase	MD39VT	*Mastotermes darwiniensis*	ND	[96]
Fungi	Xylanase	ND	*Macrotermes muelleri*	ND	[84]
	Endo-xylanase	X1T, X2T, X1Mc, X2Mc	*Macrotermes bellicosus*	ND	[97]
	Xylanase	ND	Grass-feeding termites in Thailand	GH10	[98]
	Cellulase	ND	*Macrotermitinae*	ND	[88]
	Laccase	ND	*Odontotermes formosanus*	ND	[89]
	Laccase	lcc1-2	*Microtermes* in Thailand	ND	[105]
	Cellobiohydrolases	ND	*Reticulitermes speratus*	ND	[100]

ND: not determined.

于它在中肠的酶活[20,71]，培菌白蚁后肠纤维素酶的活性也远低于中肠相应酶活[20,72–73]。对高等白蚁 *Nasutitermes corniger* 进行蛋白组学分析，发现后肠的蛋白质中缺乏与纤维素降解相关的酶，这与酶活性分析结果一致。高等白蚁后肠细菌可能类似于低等白蚁后肠细菌，产生的木质纤维素酶更多参与寡聚糖的降解。从高等食木白蚁 *Globitermes brachycerastes* 肠道菌群中鉴定到多种有功能活性的纤维二糖代谢酶，其中包括 β-葡萄糖苷酶、纤维二糖磷酸化酶、phopho-6-β-葡萄糖苷酶[10]以及木糖苷酶[74]，这一结果似乎支持上述观点。另外也从高等食木白蚁 *G. brachycerastes* 肠道微生物中获得多功能的木糖苷酶，大肠杆菌中异源表达的此木糖苷酶除具有 β-木糖苷酶活性外，还有 β-葡萄糖苷酶或 α-阿拉伯糖苷酶活性[74]。另外也有研究称从高等白蚁肠道中获得了一些新颖的酶基因[75]。在大肠杆菌中表达的共生微生物的 β-葡萄糖苷酶(bgl-gs1)为耐热的 β-葡萄糖苷酶，其最适温度达到 90 °C，在 75 °C 保温 2 h 后仍能保持 70%以上的活性。此外在大肠杆菌中重组表达的 GH11 木聚糖酶 XYL7 在 pH 5.5–10 比活性高达 6340 U/mg[76]，暗示着共生微生物来源的酶基因具有潜在的工业应用价值。

来自高等白蚁肠道细菌的木聚糖酶多含有 2 个以上结构域，木食性白蚁 *G.brachycerastes* 肠道细菌来源的木聚糖酶 Xyl-ORF19，包含 1 个 GH10 催化结构域和 1 个 Ig-like 非催化结构域[77] (表 3)。象白蚁 *Nasutitermes takasagoensis* 共生菌来源的木聚糖酶 NtSymX11，包含 1 个催化亚基、2 个碳水化合物结合亚基(CBM36)，CBM36 可以降低酶在酸性和高温条件下的稳定性，并通过增加对底物的亲和性提高酶活性[78]。

已知多数动物没有内源性木聚糖酶，它们通过共生微生物酶协助分解半纤维素。在人和牛的消化系统中，拟杆菌门 *Bacteroidetes* 通过 GH10 家族的内切木聚糖酶发挥半纤维素降解作用[79–80]，在高等象白蚁中，螺旋体菌门的 GH11 家族内切木聚糖酶发挥主要作用[9]。

关于木质素降解酶，有报道称，从高等食木白蚁 *Microtermes pakistanicus* 肠道中分离出一株具有降解木质素并能在类木质素染料培养基上快速生长的菌株 MP-4。菌株 MP-4 可能产生一些胞外酶，使木质素结构中的 S/G 比率以及联苯结构发生变化，从而降解木质素[81]。

3.3　真菌由来木质纤维素酶

相比于白蚁肠道共生细菌和原虫由来酶的研究，白蚁真菌由来木质纤维素降解酶的研究报道比较少，且主要集中在培菌白蚁方面。研究表明，培菌白蚁与鸡枞菌的共生系统能够有效降解植物材料[7,12]。早期报道白蚁通过取食菌圃中的真菌获得纤维素酶[82]，真菌产生的酶与白蚁产生的酶共同作用完成食物的消化[83]。Rouland 最早报道从培菌白蚁(*Macrotermes muelleri*)及其共生真菌中分离纯化到纤维素酶和木聚糖酶[84–85]，但是没有相关分子学方面证据。检测泰国分布的 5 种培菌白蚁菌圃及共生真菌的木质素降解酶活性，发现培菌白蚁菌圃及共生真菌都有漆酶活性，但没有检测到木质素酶和锰过氧化酶活性。鸡枞菌转录组分析鉴定了许多与植物细胞壁降解相关基因的同源序列，该数据表明鸡枞菌具有降解木质素的能力[86–87]。日本学者通过化学方法分析培菌白蚁不同年龄的菌圃组成，发现菌圃碳水化合物和木质素比率随着菌圃年龄的增加而增加，即菌圃下层木质素含量低于上层新鲜菌圃，说明共生鸡枞菌的主要作用是降解木质素，也暗示菌圃和共生菌中的漆酶与木质素降解相关[88]。从黑翅土白蚁(*Odontotermes formosanus*)菌圃纯化获得的漆酶分子量为 65 kDa，以 ABTS 为底物测得的酶活性为 211.11 U/mg[89]。

白蚁肠道除了细菌、原虫之外还有真菌，真菌可以产生多种酶。分离自桑特散白蚁肠道的两种霉菌都具有 β-葡萄糖苷酶、endo-1,4-β-D-葡聚糖酶、exo-1,4-β-D-葡聚糖酶和 endo-1,4-β-D-木聚糖酶活性，其中木聚糖酶活性最高[90]。Ali 等从散白蚁(*Reticulitermes chinenesis*)肠道中分离并鉴定了来自 18 种酵母的 92 株菌株，其中 7 种被鉴定为新物种。从白蚁肠道中分离出的共生酵母已经被证明具有产生木聚糖酶和发酵木糖的能力。共生酵母作为一个“隐身”的白蚁共生菌群在供给营养、生物质转化与利用以及抵御外来病原物方面发挥重要作用[91]。

4　总结和展望

综上所述，通过微生物分离培养以及宏转录组、宏基因组等高通量测序方法从白蚁自身、肠道细菌、真菌和体外菌圃等获得多种木质纤维素降解酶，这些酶在白蚁这个超级生命体的不同部位、不同时期发挥作用。尽管如此，围绕白蚁这个高效的超级生命体，白蚁、细菌、原生生物和真菌发生相互关系的功能基础以及白蚁肠道中发挥高效作用的核心酶类和核心微生物菌群还不是非常清楚，尤其是白蚁与体内外共生微生物之间的关系及其不同来源酶在木质纤维素降解中的协同作用和分工知之甚少。白蚁肠道细菌大多数还不能培养，但根据新的分离培养方法[101–102]，可分离之前无法培养的优势菌，从而探究其功能作用

和参与木质纤维素降解的过程或途径，了解产生的木质纤维素酶种类。另外需要扩展对不同食性更多白蚁种类的研究，揭示不同白蚁木质纤维素降解机制，挖掘更多木质纤维素酶资源。目前对半纤维素和木质素降解酶的研究主要集中于木聚糖酶和漆酶，而近年来生物信息学数据表明，白蚁自身及其共生环境中含有大量的其他相关酶序列信息，因此可以根据高通量测序数据进一步对参与半纤维素和木质素降解与修饰的辅助酶类展开研究。在此基础上，还可以通过基因工程或基因编辑方法对产生多功能木质纤维素酶菌株进行改造和酶突变筛选，从而提高目的酶活性、稳定性和产量[103]。前期我们研究发现，多种木质纤维素酶基因可以共表达，且共表达酶降解效率高于单组分混合酶[104]。基于此，在之后的研究中还可以通过遴选优良木质纤维素酶基因，人工合成构建高效表达的工程菌株，为植物基质的降解和生物质资源的转化利用奠定基础。

参考文献

[1] Tokuda G, Tsuboi Y, Kihara K, Saitou S, Moriya S, Lo N, Kikuchi J. Metabolomic profiling of ^{13}C-labelled cellulose digestion in a lower termite: insights into gut symbiont function. *Proceedings of the Royal Society B: Biological Sciences*, 2014, 281(1789): 20140990.

[2] Eggleton P. An introduction to termites: Biology, taxonomy and functional morphology//Bignell D, Roisin Y, Lo N. Biology of Termites: A Modern Synthesis. Dordrecht, The Netherlands: Springer, 2010: 1–26.

[3] Brune A. Symbiotic digestion of lignocellulose in termite guts. *Nature Reviews Microbiology*, 2014, 12(3): 168–180.

[4] Abe T, Bignell DE, Higashi T. Termites: Evolution, Sociality, Symbioses, Ecology. Netherlands: Springer, 2000.

[5] Watanabe H, Tokuda G. Cellulolytic systems in insects. *Annual Review of Entomology*, 2010, 55(1): 609–632.

[6] Sun JZ, Ding SY, Doran-Peterson J. Biomass and its biorefinery: novel approaches from nature-inspired strategies and technology//Sun JZ, Ding SY, Doran-Peterson J. Biological Conversion of Biomass for Fuels and Chemicals: Explorations from Natural Utilization Systems. Cambridge, UK: RSC Publishing, 2013.

[7] Li HJ, Yelle DJ, Li C, Yang MY, Ke J, Zhang RJ, Liu Y, Zhu N, Liang SY, Mo XC, Ralph J, Currie CR, Mo JC. Lignocellulose pretreatment in a fungus-cultivating termite. *Proceedings of the National Academy of Sciences of the United States of America*, 2017, 114(18): 4709–4714.

[8] Brune A. Termite guts: The world's smallest bioreactors. *Trends in Biotechnology*, 1998, 16(1): 16–21.

[9] Tokuda G, Mikaelyan A, Fukui C, Matsuura Y, Watanabe H, Fujishima M, Brune A. Fiber-associated spirochetes are major agents of hemicellulose degradation in the hindgut of wood-feeding higher termites. *Proceedings of the National Academy of Sciences of the United States of America*, 2018, 115(51): E11996–E12004.

[10] Liu N, Li HJ, Chevrette MG, Zhang L, Cao L, Zhou HK, Zhou XG, Zhou ZH, Pope PB, Currie CR, Huang YP, Wang Q. Functional metagenomics reveals abundant polysaccharide-degrading gene clusters and cellobiose utilization pathways within gut microbiota of a wood-feeding higher termite. *The ISME Journal*, 2019, 13(1): 104–117.

[11] Poulsen M, Hu HF, Li C, Chen ZS, Xu LH, Otani S, Nygaard S, Nobre T, Klaubauf S, Schindler PM, Hauser F, Pan HL, Yang ZK, Sonnenberg ASM, de Beer ZW, Zhang Y, Wingfield MJ, Grimmelikhuijzen CJP, de Vries RP, Korb J, Aanen DK, Wang J, Boomsma JJ, Zhang GJ. Complementary symbiont contributions to plant decomposition in a fungus-farming termite. *Proceedings of the National Academy of Sciences of the United States of America*, 2014, 111(40): 14500–14505.

[12] da Costa RR, Hu HF, Li HJ, Poulsen M. Symbiotic plant biomass decomposition in fungus-growing termites. *Insects*, 2019, 10(4): 87.

[13] Inward D, Beccaloni G, Eggleton P. Death of an order: a comprehensive molecular phylogenetic study confirms that termites are eusocial cockroaches. *Biology Letters*, 2007, 3(3): 331–335.

[14] Um S, Fraimout A, Sapountzis P, Oh DC, Poulsen M. The fungus-growing termite *Macrotermes natalensis* harbors bacillaene-producing *Bacillus* sp. that inhibit potentially antagonistic fungi. *Scientific Reports*, 2013, 3: 3250.

[15] Aanen DK, Eggleton P, Rouland-Lefevre C, Guldberg-Frøslev T, Rosendahl S, Boomsma JJ. The evolution of fungus-growing termites and their mutualistic fungal symbionts. *Proceedings of the National Academy of Sciences of the United States of America*, 2002, 99(23): 14887–14892.

[16] Hager FA, Kirchner WH. Vibrational long-distance communication in the termites *Macrotermes natalensis* and *Odontotermes* sp.. *Journal of Experimental Biology*, 2013, 216(17): 3249–3256.

[17] Ramadhar TR, Beemelmanns C, Currie CR, Clardy J. Bacterial symbionts in agricultural systems provide a strategic source for antibiotic discovery. *The Journal of Antibiotics*, 2014, 67(1): 53–58.

[18] Aanen DK, Eggleton P. Fungus-growing termites originated in African rain forest. *Current Biology*, 2005, 15(9): 851–855.

[19] Tokuda G, Watanabe H, Matsumoto T, Noda H. Cellulose digestion in the wood-eating higher termite, *Nasutitermes takasagoensis* (Shiraki): distribution of cellulases and properties of endo-β-1, 4-glucanase. *Zoological Science*, 1997, 14(1): 83–93.

[20] Ni JF, Tokuda G. Lignocellulose-degrading enzymes from termites and their symbiotic microbiota. *Biotechnology Advances*, 2013, 31(6): 838–850.

[21] Polizeli MLTM, Rizzatti ACS, Monti R, Terenzi HF, Jorge JA, Amorim DS. Xylanases from fungi: properties and industrial applications. *Applied Microbiology and Biotechnology*, 2005, 67(5): 577–591.

[22] Wong KK, Tan LU, Saddler JN. Multiplicity of beta-1,4-xylanase in microorganisms: functions and applications. *Microbiological Reviews*, 1988, 52(3): 305–317.

[23] Himmel ME, Ding SY, Johnson DK, Adney WS, Nimlos MR, Brady JW, Foust TD. Biomass recalcitrance: engineering plants and enzymes for biofuels production. *Science*, 2007, 315(5813): 804–807.

[24] Watanabe H, Noda H, Tokuda G, Lo N. A cellulase gene of termite origin. *Nature*, 1998, 394(6691): 330–331.

[25] Ni JF, Wu Y, Yun C, Yu ML, Shen YL. cDNA cloning and heterologous expression of an endo-β-1,4-glucanase from the fungus-growing termite *Macrotermes barneyi. Archives of Insect Biochemistry and Physiology*, 2014, 86(3): 151–164.

[26] Arthornthurasuk S, Jenkhetkan W, Suwan E, Chokchaichamnankit D, Srisomsap C, Wattana-Amorn P, Svasti J, Kongsaeree PT. Molecular characterization and potential synthetic applications of GH1 β-glucosidase from higher termite *Microcerotermes annandalei*. *Applied Biochemistry and Biotechnology*, 2018, 186(4): 877–894.

[27] Shimada K, Maekawa K. Gene expression and molecular phylogenetic analyses of beta-glucosidase in the termite *Reticulitermes speratus* (Isoptera: Rhinotermitidae). *Journal of Insect Physiology*, 2014, 65: 63–69.

[28] Zhang PF, Yuan XH, Du YG, Li JJ. Heterologous expression and biochemical characterization of a GHF9 endoglucanase from the termite *Reticulitermes speratus* in *Pichia pastoris*. *BMC Biotechnology*, 2018, 18(1): 35.

[29] Ni JF, Takehara M, Miyazawa M, Watanabe H. Random exchanges of non-conserved amino acid residues among four parental termite cellulases by family shuffling improved thermostability. *Protein Engineering, Design & Selection*, 2007, 20(11): 535–542.

[30] Hirayama K, Watanabe H, Tokuda G, Kitamoto K, Arioka M. Purification and characterization of termite endogenous β-1,4-endoglucanases produced in *Aspergillus oryzae*. *Bioscience, Biotechnology, and Biochemistry*, 2010, 74(8): 1680–1686.

[31] Wu WJ, Li ZQ. dsRNA injection successfully inhibited two endogenous β-glucosidases in *Coptotermes formosanus* (*Isoptera*: *Rhinotermitidae*). *Journal of Economic Entomology*, 2018, 111(2): 860–867.

[32] Wu WJ, Gu DF, Yan SC, Li ZQ. RNA interference of endoglucanases in the formosan subterranean termite *Coptotermes formosanus shiraki* (*Blattodea*: *Rhinotermitidae*) by dsRNA injection or ingestion. *Journal of Insect Physiology*, 2019, 112: 15–22.

[33] Coy MR, Salem TZ, Denton JS, Kovaleva ES, Liu Z, Barber DS, Campbell JH, Davis DC, Buchman GW, Boucias DG, Scharf ME. Phenol-oxidizing laccases from the termite gut. *Insect Biochemistry and Molecular Biology*, 2010, 40(10): 723–732.

[34] Tartar A, Wheeler MM, Zhou XG, Coy MR, Boucias DG, Scharf ME. Parallel metatranscriptome analyses of host and symbiont gene expression in the gut of the termite *Reticulitermes flavipes*. *Biotechnology for Biofuels*, 2009, 2: 25.

[35] Geng AL, Wu J, Xie RR, Li X, Chang FX, Sun JZ. Characterization of a laccase from a wood-feeding termite, *Coptotermes formosanus*. *Insect Science*, 2018, 25(2): 251–258.

[36] Watanabe H, Nakamura M, Tokuda G, Yamaoka I, Scrivener AM, Noda H. Site of secretion and properties of endogenous

endo-β-1,4-glucanase components from *Reticulitermes speratus* (Kolbe): a Japanese subterranean termite. *Insect Biochemistry and Molecular Biology*, 1997, 27(4): 305–313.

[37] Khademi S, Guarino LA, Watanabe H, Tokuda G, Meyer EF. Structure of an endoglucanase from termite, *Nasutitermes takasagoensis*. *Acta Crystallographica. Section D, Biological Crystallography*, 2002, 58(4): 653–659.

[38] Zhang DH, Lax AR, Raina AK, Bland JM. Differential cellulolytic activity of native-form and C-terminal tagged-form cellulase derived from *Coptotermes formosanus* and expressed in *E. coli*. *Insect Biochemistry and Molecular Biology*, 2009, 39(8): 516–522.

[39] Ni JF, Takehara M, Watanabe H. Identification of activity related amino acid mutations of a GH9 termite cellulase. *Bioresource Technology*, 2010, 101(16): 6438–6443.

[40] Tokuda G, Saito H, Watanabe H. A digestive β-glucosidase from the salivary glands of the termite, *Neotermes koshunensis* (Shiraki): distribution, characterization and isolation of its precursor cDNA by 5′- and 3′-RACE amplifications with degenerate primers. *Insect Biochemistry and Molecular Biology*, 2002, 32(12): 1681–1689.

[41] Xue YP, Jin LQ, Liu ZQ, Zhang JF, Zheng YG. Purification and characterization of β-glucosidase from *Reticulitermes flaviceps* and its inhibition by valienamine and validamine. *African Journal of Biotechnology*, 2008, 7(24): 4595–4601.

[42] Scharf ME, Kovaleva ES, Jadhao S, Campbell JH, Buchman GW, Boucias DG. Functional and translational analyses of a beta-glucosidase gene (glycosyl hydrolase family 1) isolated from the gut of the lower termite *Reticulitermes flavipes*. *Insect Biochemistry and Molecular Biology*, 2010, 40(8): 611–620.

[43] Mattéotti C, Haubruge E, Thonart P, Francis F, De Pauw E, Portetelle D, Vandenbol M. Characterization of a new β-glucosidase/βa-xylosidase from the gut microbiota of the termite (*Reticulitermes santonensis*). *FEMS Microbiology Letters*, 2011, 314(2): 147–157.

[44] Uchima CA, Tokuda G, Watanabe H, Kitamoto K, Arioka M. Heterologous expression in Pichia pastoris and characterization of an endogenous thermostable and high-glucose-tolerant β-glucosidase from the termite *Nasutitermes takasagoensis*. *Applied and Environmental Microbiology*, 2012, 78(12): 4288–4293.

[45] Uchima CA, Tokuda G, Watanabe H, Kitamoto K, Arioka M. Heterologous expression and characterization of a glucose-stimulated β-glucosidase from the termite *Neotermes koshunensis* in *Aspergillus oryzae*. *Applied Microbiology and Biotechnology*, 2011, 89(6): 1761–1771.

[46] Jeng WY, Wang NC, Lin CT, Chang WJ, Liu CI, Wang AH. High-resolution structures of *Neotermes koshunensis* β-glucosidase mutants provide insights into the catalytic mechanism and the synthesis of glucoconjugates. *Acta Crystallographica. Section D, Biological Crystallography*, 2012, 68(7): 829–838.

[47] Wu Y, Chi S, Yun C, Shen Y, Tokuda G, Ni J. Molecular cloning and characterization of an endogenous digestive beta-glucosidase from the midgut of the fungus-growing termite *Macrotermes barneyi*. *Insect Molecular Biology*, 2012, 21(6): 604–614.

[48] Zhang ML, Liu N, Qian CL, Wang QF, Wang Q, Long YH, Huang YP, Zhou ZH, Yan X. Phylogenetic and functional analysis of gut microbiota of a fungus-growing higher termite: bacteroidetes from higher termites are a rich source of β-glucosidase genes. *Microbial Ecology*, 2014, 68(2): 416–425.

[49] Yuki M, Moriya S, Inoue T, Kudo T. Transcriptome analysis of the digestive organs of *Hodotermopsis sjostedti*, a lower termite that hosts mutualistic microorganisms in its hindgut. *Zoological Science*, 2008, 25(4): 401–406.

[50] Zhang D, Lax AR, Henrissat B, Coutinho P, Katiya N, Nierman WC, Fedorova N. Carbohydrate-active enzymes revealed in *Coptotermes formosanus* (*Isoptera*: *Rhinotermitidae*) transcriptome. *Insect Molecular Biology*, 2012, 21(2): 235–245.

[51] Todaka N, Moriya S, Saita K, Hondo T, Kiuchi I, Takasu H, Ohkuma M, Piero C, Hayashizaki Y, Kudo T. Environmental cDNA analysis of the genes involved in lignocellulose digestion in the symbiotic protist community of *Reticulitermes speratus*. *FEMS Microbiology Ecology*, 2007, 59(3): 592–599.

[52] Geng AL, Cheng YB, Wang YL, Zhu DC, Le YL, Wu J, Xie RR, Yuan JS, Sun JZ. Transcriptome analysis of the digestive system of a wood-feeding termite (*Coptotermes formosanus*) revealed a unique mechanism for effective biomass degradation. *Biotechnology for Biofuels*, 2018, 11: 24.

[53] Xie L, Zhang L, Zhong Y, Liu N, Long YH, Wang SY, Zhou XG, Zhou ZH, Huang YP, Wang Q. Profiling the metatranscriptome of the protistan community in *Coptotermes formosanus* with emphasis on the lignocellulolytic system.

Genomics, 2012, 99(4): 246–255.

[54] Cho MJ, Kim YH, Shin K, Kim YK, Kim YS, Kim TJ. Symbiotic adaptation of bacteria in the gut of *Reticulitermes speratus*: low endo-β-1,4-glucanase activity. *Biochemical and Biophysical Research Communications*, 2010, 395(3): 432–435.

[55] Treitli SC, Kolisko M, Husnik F, Keeling PJ, Hampl V. Revealing the metabolic capacity of *Streblomastix strix* and its bacterial symbionts using single-cell metagenomics. *Proceedings of the National Academy of Sciences of the United States of America*, 2019, 116(39): 19675–19684.

[56] Sethi A, Slack JM, Kovaleva ES, Buchman GW, Scharf ME. Lignin-associated metagene expression in a lignocellulose-digesting termite. *Insect Biochemistry and Molecular Biology*, 2013, 43(1): 91–101.

[57] Liu XJ, Xie L, Liu N, Zhan S, Zhou XG, Wang Q. RNA interference unveils the importance of *Pseudotrichonympha grassii* cellobiohydrolase, a protozoan exoglucanase, in termite cellulose degradation. *Insect Molecular Biology*, 2017, 26(2): 233–242.

[58] Arakawa G, Watanabe H, Yamasaki H, Maekawa H, Tokuda G. Purification and molecular cloning of xylanases from the wood-feeding termite, *Coptotermes formosanus* Shiraki. *Bioscience, Biotechnology, and Biochemistry*, 2009, 73(3): 710–718.

[59] Mattéotti C, Bauwens J, Brasseur C, Tarayre C, Thonart P, Destain J, Francis F, Haubruge E, De Pauw E, Portetelle D, Vandenbol M. Identification and characterization of a new xylanase from Gram-positive bacteria isolated from termite gut (*Reticulitermes santonensis*). *Protein Expression and Purification*, 2012, 83(2): 117–127.

[60] Watanabe H, Nakashima K, Saito H, Slaytor M. New endo-β-1,4-glucanases from the parabasalian symbionts, *Pseudotrichonympha grassii* and *Holomastigotoides mirabile* of *Coptotermes termites*. *Cellular and Molecular Life Sciences*, 2002, 59(11): 1983–1992.

[61] Li L, Fröhlich J, Pfeiffer P, König H. Termite gut symbiotic archaezoa are becoming living metabolic fossils. *Eukaryotic Cell*, 2003, 2(5): 1091–1098.

[62] Inoue T, Moriya S, Ohkuma M, Kudo T. Molecular cloning and characterization of a cellulase gene from a symbiotic protist of the lower termite, *Coptotermes formosanus*. *Gene*, 2005, 349: 67–75.

[63] Todaka N, Lopez CM, Inoue T, Saita K, Maruyama J, Arioka M, Kitamoto K, Kudo T, Moriya S. Heterologous expression and characterization of an endoglucanase from a symbiotic protist of the lower termite, *Reticulitermes speratus*. *Applied Biochemistry and Biotechnology*, 2010, 160(4): 1168–1178.

[64] Otagiri M, Lopez CM, Kitamoto K, Arioka M, Kudo T, Moriya S. Heterologous expression and characterization of a glycoside hydrolase family 45 endo-β-1,4-Glucanase from a symbiotic protist of the lower termite, *Reticulitermes speratus*. *Applied Biochemistry and Biotechnology*, 2013, 169(6): 1910–1918.

[65] Nakashima K, Watanabe H, Azuma JI. Cellulase genes from the parabasalian symbiont *Pseudotrichonympha grassii* in the hindgut of the wood-feeding termite *Coptotermes formosanus*. *Cellular and Molecular Life Sciences*, 2002, 59(9): 1554–1560.

[66] Ohtoko K, Ohkuma M, Moriya S, Inoue T, Usami R, Kudo T. Diverse genes of cellulase homologues of glycosyl hydrolase family 45 from the symbiotic protists in the hindgut of the termite *Reticulitermes speratus*. *Extremophiles*, 2000, 4(6): 343–349.

[67] Zhou XG, Smith JA, Oi FM, Koehler PG, Bennett GW, Scharf ME. Correlation of cellulase gene expression and cellulolytic activity throughout the gut of the termite *Reticulitermes flavipes*. *Gene*, 2007, 395(1/2): 29–39.

[68] Warnecke F, Luginbühl P, Ivanova N, Ghassemian M, Richardson TH, Stege JT, Cayouette M, McHardy AC, Djordjevic G, Aboushadi N, Sorek R, Tringe SG, Podar M, Martin HG, Kunin V, Dalevi D, Madejska J, Kirton E, Platt D, Szeto E, Salamov A, Barry K, Mikhailova N, Kyrpides NC, Matson EG, Ottesen EA, Zhang XN, Hernández M, Murillo C, Acosta LG, Rigoutsos I, Tamayo G, Green BD, Chang C, Rubin EM, Mathur EJ, Robertson DE, Hugenholtz P, Leadbetter JR. Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature*, 2007, 450(7169): 560–565.

[69] Liu N, Yan X, Zhang ML, Xie L, Wang Q, Huang YP, Zhou XG, Wang SY, Zhou ZH. Microbiome of fungus-growing termites: a new reservoir for lignocellulase genes. *Applied and Environmental Microbiology*, 2011, 77(1): 48–56.

[70] Rashamuse K, Sanyika Tendai W, Mathiba K, Ngcobo T, Mtimka S, Brady D. Metagenomic mining of glycoside hydrolases from the hindgut bacterial symbionts of a termite (*Trinervitermes trinervoides*) and the characterization of a multimodular β-1,4-xylanase (GH11). *Biotechnology and*

Applied Biochemistry, 2017, 64(2): 174–186.

[71] Tokuda G, Lo N, Watanabe H, Arakawa G, Matsumoto T, Noda H. Major alteration of the expression site of endogenous cellulases in members of an apical termite lineage. *Molecular Ecology*, 2004, 13(10): 3219–3228.

[72] Li ZQ, Liu BR, Zeng WH, Xiao WL, Li QJ, Zhong JH. Character of cellulase activity in the guts of flagellate-free termites with different feeding habits. *Journal of Insect Science*, 2013, 13(37): 1–8.

[73] Crosland MWJ, Chan LK, Buswell JA. Symbiotic fungus and enzymatic digestion in the gut of the termite, *Macrotermes barneyi* (Light) (*Isoptera*: *Termitidae*). *Journal of Entomological Science*, 1996, 31(1): 132–137.

[74] Liu CY, Zou G, Yan X, Zhou XG. Screening of multimeric β-xylosidases from the gut microbiome of a higher termite, *Globitermes brachycerastes*. *International Journal of Biological Sciences*, 2018, 14(6): 608–615.

[75] Wang QF, Qian CL, Zhang XZ, Liu N, Yan X, Zhou ZH. Characterization of a novel thermostable β-glucosidase from a metagenomic library of termite gut. *Enzyme and Microbial Technolog*, 2012, 51(6/7): 319–324.

[76] Qian CL, Liu N, Yan X, Wang Q, Zhou ZH, Wang QF. Engineering a high-performance, metagenomic-derived novel xylanase with improved soluble protein yield and thermostability. *Enzyme and Microbial Technology*, 2015, 70: 35–41.

[77] Han Q, Liu N, Robinson H, Cao L, Qian CL, Wang QF, Xie L, Ding HZ, Wang Q, Huang YP, Li JY, Zhou ZH. Biochemical characterization and crystal structure of a GH10 xylanase from termite gut bacteria reveal a novel structural feature and significance of its bacterial Ig-like domain. *Biotechnology and Bioengineering*, 2013, 110(12): 3093–3103.

[78] Kitamoto M, Tokuda G, Watanabe H, Arioka M. Characterization of CBM36-containing GH11 endoxylanase NtSymX11 from the hindgut metagenome of higher termite *Nasutitermes takasagoensis* displaying prominent catalytic activity. *Carbohydrate Research*, 2019, 474: 1–7.

[79] Dodd D, Mackie RI, Cann IKO. Xylan degradation, a metabolic property shared by rumen and human colonic Bacteroidetes. *Molecular Microbiology*, 2011, 79(2): 292–304.

[80] Zhang ML, Chekan JR, Dodd D, Hong PY, Radlinski L, Revindran V, Nair SK, Mackie RI, Cann I. Xylan utilization in human gut commensal bacteria is orchestrated by unique modular organization of polysaccharide-degrading enzymes. *Proceedings of the National Academy of Sciences of the United States of America*, 2014, 111(35): E3708–E3717.

[81] Li F, Xie R, Liang N, Sun JZ, Zhu DC. Biodegradation of lignin via *Pseudocitrobacter anthropi* MP-4 isolated from the gut of wood-feeding termite *Microtermes pakistanicus* (Isoptera: termitidae). *BioResources*, 2019, 14(1): 1992–2012.

[82] Martin MM, Martin JS. Cellulose digestion in the midgut of the fungus-growing termite *Macrotermes natalensis*: the role of acquired digestive enzymes. *Science*, 1978, 199(4336): 1453–1455.

[83] Rouland C, Lenoir F, Lepage M. The role of the symbiotic fungus in the digestive metabolism of several species of fungus-growing termites. *Comparative Biochemistry & Physiology Part A: Physiology*, 1991, 99(4): 657–663.

[84] Rouland C, Renoux J, Petek F. Purification and properties of two xylanases from *Macrotermes mülleri* (Termitidae: Macrotermitinae) and its symbiotic fungus *Termitomyces* sp. *Insect Biochemistry*, 1988, 18(7): 709–715.

[85] Rouland C, Civas A, Renoux J, Petek F. Purification and properties of cellulases from the termite *Macrotermes mülleri* (Termitidae, Macrotermitinae) and its symbiotic fungus *Termitomyces* sp. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 1988, 91(3): 449–458.

[86] Johjima T, Taprab Y, Noparatnaraporn N, Kudo T, Ohkuma M. Large-scale identification of transcripts expressed in a symbiotic fungus (Termitomyces) during plant biomass degradation. *Applied Microbiology and Biotechnology*, 2006, 73(1): 195–203.

[87] Yang F, Xu B, Li JJ, Huang ZX. Transcriptome analysis of *Termitomyces albuminosus* reveals the biodegradation of lignocellulose. *Acta Microbiologica Sinica*, 2012, 52(4): 466–477. (in Chinese)
杨芳, 许波, 李俊俊, 黄遵锡. 鸡枞菌转录组分析揭示其对木质纤维素的降解功能. 微生物学报, 2012, 52(4): 466–477.

[88] Hyodo F, Tayasu I, Inoue T, Azuma JI, Kudo T, Abe T. Differential role of symbiotic fungi in lignin degradation and food provision for fungus-growing termites (Macrotermitinae: Isoptera). *Functional Ecology*, 2003, 17(2): 186–193.

[89] Zhou Y, Deng TF, Pan CY, Chen CR, Mo JC. Purification of a laccase from fungus combs in the nest of Odontotermes formosanus. *Process Biochemistry*, 2010, 45(7): 1052–1056.

[90] Tarayre C, Bauwens J, Brasseur C, Mattéotti C, Millet C,

Guiot PA, Destain J, Vandenbol M, Portetelle D, De Pauw E, Haubruge E, Francis F, Thonart P. Isolation and cultivation of xylanolytic and cellulolytic *Sarocladium kiliense* and *Trichoderma virens* from the gut of the termite *Reticulitermes santonensis*. *Environmental Science and Pollution Research*, 2015, 22(6): 4369–4382.

[91] Ali SS, Wu J, Xie RR, Zhou F, Sun JZ, Huang M. Screening and characterizing of xylanolytic and xylose-fermenting yeasts isolated from the wood-feeding termite, *Reticulitermes chinensis*. *PLoS One*, 2017, 12(7): e0181141.

[92] Bai X, Yuan XJ, Wen AY, Li JF, Bai YF, Shao T. Cloning, expression and characterization of a cold-adapted endo-1, 4-β-glucanase from *Citrobacter farmeri* A1, a symbiotic bacterium of *Reticulitermes labralis*. *PeerJ*, 2016, 4: e2679.

[93] Javaheri-Kermani M, Asoodeh A. A novel beta-1, 4 glucanase produced by symbiotic *Bacillus* sp. CF96 isolated from termite (Anacanthotermes). *International Journal of Biological Macromolecules*, 2019, 131: 752–759.

[94] Dheeran P, Nandhagopal N, Kumar S, Jaiswal YK, Adhikari DK. A novel thermostable xylanase of *Paenibacillus macerans* IIPSP3 isolated from the termite gut. *Journal of Industrial Microbiology & Biotechnology*, 2012, 39(6): 851–860.

[95] Tsegaye B, Balomajumder C, Roy P. Isolation and characterization of novel lignolytic, cellulolytic, and hemicellulolytic bacteria from wood-feeding termite *Cryptotermes brevis*. *International Microbiology*, 2019, 22(1): 29–39.

[96] Eugenio M, Müller N, Frasés S, Almeida-Paes R, Lima LMTR, Lemgruber L, Farina M, de Souza W, Sant'Anna C. Yeast-derived biosynthesis of silver/silver chloride nanoparticles and their antiproliferative activity against bacteria. *RSC Advances*, 2016, 6(12): 9893–9904.

[97] Matoub M, Rouland C. Purification and properties of the xylanases from the termite *Macrotermes bellicosus* and its symbiotic fungus *Termitomyces* sp. *Comparative biochemistry and physiology. Part B: Biochemistry & Molecular Biology*, 1995, 112(4): 629–635.

[98] Sinma K, Khucharoenphaisan K, Kitpreechavanich V, Tokuyama S. Purification and characterization of a thermostable xylanase from *Saccharopolyspora pathumthaniensis* S582 isolated from the gut of a termite. *Bioscience, Biotechnology, and Biochemistry*, 2011, 75(10): 1957–1963.

[99] Zhou XG, Kovaleva ES, Wu-Scharf D, Campbell JH, Buchman GW, Boucias DG, Scharf ME. Production and characterization of a recombinant beta-1,4-endoglucanase (glycohydrolase family 9) from the termite Reticulitermes flavipes. *Archives of Insect Biochemistry and Physiology*, 2010, 74(3): 147–162.

[100] Lee CC, Lee CY. A laboratory maintenance regime for a fungus-growing termite *Macrotermes gilvus* (*Blattodea*: *Termitidae*). *Journal of Economic Entomology*, 2015, 108(3): 1243–1250.

[101] Mei C, Fan S, Yang H. The strategies of isolation of insect gut microorganisms. *Acta Microbiologica Sinica*, 2018, 58(6): 985–994. (in Chinese)
梅承, 范硕, 杨红. 昆虫肠道微生物分离培养策略及研究进展. 微生物学报, 2018, 58(6): 985–994.

[102] Cross KL, Campbell JH, Balachandran M, Campbell AG, Cooper SJ, Griffen A, Heaton M, Joshi S, Klingeman D, Leys E, Yang ZM, Parks JM, Podar M. Targeted isolation and cultivation of uncultivated bacteria by reverse genomics. *Nature Biotechnology*, 2019, 37(11): 1314–1321.

[103] Liu Q, Gao RR, Li JG, Lin LC, Zhao JQ, Sun WL, Tian CG. Development of a genome-editing CRISPR/Cas9 system in thermophilic fungal *Myceliophthora species* and its application to hyper-cellulase production strain engineering. *Biotechnology for Biofuels*, 2017, 10: 1.

[104] Du J, Jiang SJ, Wei JH, Shen YL, Ni JF. Co-expression of lignocellulase from termite and their endosymbionts. *Chinese Journal of Biotechnology*, 2019, 35(2): 244–253. (in Chinese)
杜娇, 姜淑喆, 未建华, 申玉龙, 倪金凤. 白蚁及其共生菌来源的 4 种木质纤维素酶的共表达. 生物工程学报, 2019, 35(2): 244–253.

[105] Taprab Y, Johjima T, Maeda Y, Moriya S, Trakulnaleamsai S, Noparatnaraporn N, Ohkuma M, Kudo T. Symbiotic fungi produce laccases potentially involved in phenol degradation in fungus combs of fungus-growing termites in Thailand. *Applied and Environmental Microbiology*, 2005, 71(12): 7696–7704.

Advances in lignocellulose-degrading enzymes from termites and symbiotic microbes

Yutong Jiang, Shuo Zhang, Zijia Lin, Jinfeng Ni*

State Key Laboratory of Microbial Technology, Shandong University, Qingdao 266237, Shandong Province, China

Abstract: Lignocellulose is the most abundant organic polymer on earth. Termites are one of the oldest but most successful and efficient lignocellulose degraders. Understanding the mechanism of effective degradation of highly resistant plant polymers by termites has important guidance for industrial biomass energy conversion and biomimetic design. The lignocellulases produced by termites and their symbiotic microorganisms play an important role in the conversion and utilization of lignocellulose. This article summarizes the research progress in producing cellulase, xylanase and laccase by termites themselves and their intestinal protozoa, bacteria and fungi, and suggests future problems and prospects. This review is helpful for a comprehensive understanding of the lignocellulytic gene type, source, distribution, expression, and enzyme activity and function of lignocellulases in termite digestive system.

Keywords: termites, lignocellulose degradation, cellulose, xylanase, laccase

（本文责编：张晓丽）

Supported by the National Natural Science Foundation of China (31970119, 31272370)

*Corresponding author. Tel/Fax: +86-532-58631564; E-mail: jinfgni@sdu.edu.cn

Received: 30 October 2019; Revised: 8 January 2020; Published online: 9 November 2020

倪金凤，山东大学微生物技术研究院/微生物技术国家重点实验室教授，博士生导师。曾任日本农业生物资源研究所助理，日本学术振兴会外国人特别研究员，韩国首尔大学生化工程系访问教授；是中国昆虫微生物组和中国城市昆虫专业委员会委员；国家自然科学基金项目评审专家，国内外相关学术期刊审稿专家。主要研究领域为特殊环境（极端环境和白蚁肠道）微生物组及木质纤维素资源的高效降解与利用。主持或参与了国家自然科学基金重点项目、面上项目以及国家重点基础研究发展计划子课题等多个项目，在 *Biotechnology Advance*，*Bioresource Technology*，*Appl Biochem Biotech*，*Insect Mol Biol*，*Insect Science*，*Int J Syst Evol Microbiol*，*Biosci Biotechnol Biochem* 等国际学术期刊和国内核心学报期刊发表相关论文 50 余篇。