



# 微生物降解秸秆木质素的研究进展

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**摘要:** 我国秸秆资源丰富, 每年产生逾 8 亿 t 作物秸秆。通过秸秆直接还田或肥料化还田不仅可以减少化肥的施用量, 缓解农业污染压力, 还能实现农作物秸秆的循环利用。木质素结构复杂, 且与纤维素和半纤维素相互缠绕, 因此秸秆的自然腐解过程中, 木质素是主要的限速因子, 为了提高降解效率, 木质素降解菌的发掘和降解机制也逐渐成为研究热点。本文综述了降解木质素的真菌和细菌的研究现状, 对比其真菌和细菌降解特性的优缺点并分析复合降解菌群的优势。随后对木质素降解酶系的酶学性质、在不同微生物中的表达特性进行总结, 对木质素降解机制及衍生芳烃代谢路径的研究进展进行综述。最后整理木质素降解微生物在秸秆肥料化技术中的应用进展, 并探讨了微生物降解秸秆木质素的应用前景和未来的研究方向。

**关键词:** 秸秆; 木质素; 微生物降解; 木质素降解酶; 生物降解机制

## Research progress in microbial degradation of straw lignin

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**Abstract:** China has abundant straw resources, with over 800 million tons of crop straw

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produced annually. Directly returning straw to farmlands or using it as fertilizer can not only reduce the use of chemical fertilizers and alleviate agricultural non-point source pollution but also achieve the recycling of crop straw. Lignin has a complex structure and is entangled with cellulose and hemicellulose. Therefore, in the natural decomposition process of straw, lignin is the main limiting factor. Mining lignin-degrading microorganisms and deciphering their degradation mechanisms to improve the degradation efficiency have gradually become research hotspots. By reviewing the available studies of lignin-degrading microorganisms, we compared the lignin degradation characteristics of fungi and bacteria and analyzed the advantages of composite degrading consortia. Subsequently, we summarized the properties of lignin-degrading enzymes and their expression characteristics in different microorganisms, and introduced the research progress in lignin degradation mechanisms and derived aromatic hydrocarbon metabolic pathways. Finally, we reviewed the application of lignin-degrading microorganisms in the production of straw fertilizers and put forward the application prospects and research directions of microbial degradation of lignin in straw.

**Keywords:** straw; lignin; microbial degradation; lignin-degrading enzymes; mechanism of bio-degradation

我国作为农业大国, 每年产生大量的农作物秸秆。全国农作物秸秆综合利用情况报告显示, 2021年, 我国农作物秸秆利用量为 6.47 亿 t, 综合利用率达 88.1%, 其中秸秆肥料化量达 4 亿 t, 占利用量的 61.8%<sup>[1]</sup>。农作物收获后残留的秸秆中含有丰富的碳、氮、磷等大量元素和作物生长所需要的中微量元素, 秸秆肥料化还田不仅能够提高土壤肥力, 减少化肥的施用量, 还能实现秸秆资源循环利用。但在实际应用过程中, 秸秆肥料化技术中还存在较为突出的秸秆降解效率偏低的问题, 这与秸秆天然的结构特征有关。

秸秆成分一般主要包括纤维素(占 35%–50%)、半纤维素(占 20%–35%)和木质素(占 15%–25%), 其他成分还包括微量的果胶、氮化合物和无机成分<sup>[2-3]</sup>。其中木质素嵌入纤维素和半纤维素结构之间, 形成复杂的异质网络, 限制了微生物纤维

素酶和半纤维素酶的可及性。因此, 木质素是环境微生物降解秸秆的主要屏障<sup>[4]</sup>。木质素具有高度交联的三维网状结构<sup>[5-7]</sup>, 一般由 3 种苯基丙烷结构单体——愈创木基(G 型)、紫丁香基(S 型)和对羟基苯基(H 型) (图 1A)通过不同类型的醚键和碳碳(C–C)键无序聚合形成(图 1B)。在不同类型的木质素中 3 种单体的组合和比例也存在差异。研究表明, 硬木木质素由 S 和 G 单体组成, 软木木质素由 G 单体和少量 H 单体组成, 而包括玉米、水稻和小麦等秸秆在内的草本木质素则包括所有 3 种单体<sup>[8-9]</sup>。由于木质素的天然降解十分缓慢, 为了提高秸秆腐熟效率, 木质素高效降解菌的发掘成为近年来研究的热点。本文综述了近年木质素降解菌、降解酶和降解机制的研究进展, 并讨论了秸秆木质素降解菌的应用前景和未来的研究方向, 以期后续研究以及秸秆高效利用提供理论基础。

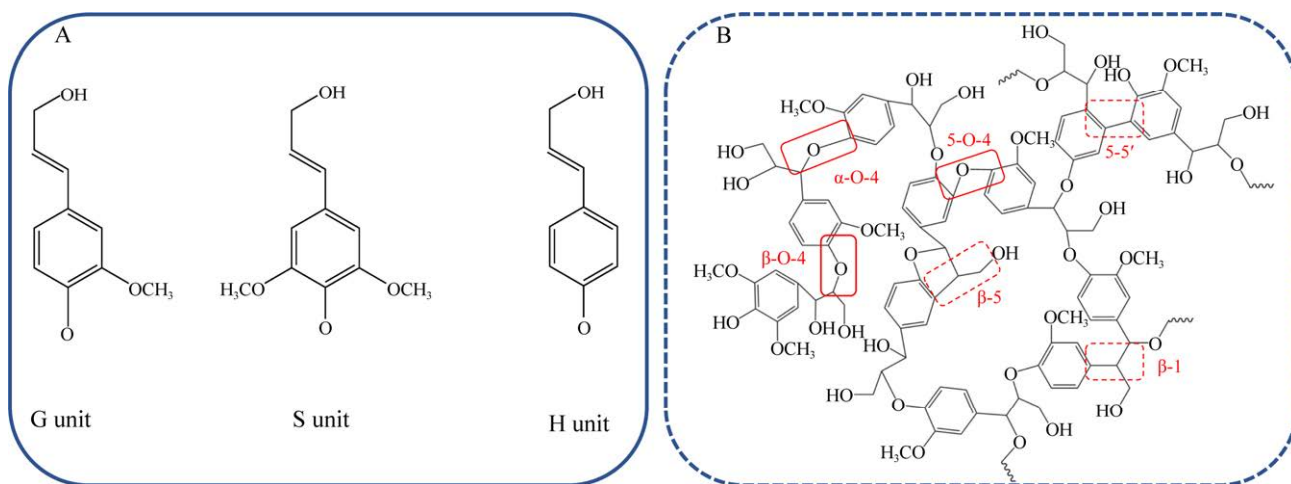


图 1 木质素结构特征<sup>[5-7]</sup>

Figure 1 Lignin structural features<sup>[5-7]</sup>. A: The three phenyl propane monomers in the lignin structure. B: The lignin structure with different types of carbon-carbon bonds (red dashed box) and ether bonds (red solid box) linked to the disordered polymerization of lignin monomers. The carbon-carbon bonds in the lignin structure mainly include  $\beta$ -1 (1,2-diaryl-propane bond),  $\beta$ -5 (phenyl coumaranbond), and 5-5 (diaryl) bond, and the ether bonds mainly include  $\alpha$ -O-4 (alkyl-aryl ether) bond,  $\beta$ -O-4 (alkyl-aryl ether) bond and 4-O-5 (diaryl ether) bond. Among them, the  $\beta$ -O-4 bond is the most abundant, accounting for about 50% of the structural bonds of lignin.

## 1 秸秆木质素降解菌的发掘现状

相比物理化学方法,利用微生物降解木质素是对环境更为友好的一种提高降解效率的技术手段。目前报道中仅白腐真菌(white-rot fungi)这类微生物能够将木质素完全降解为  $\text{CO}_2$  和  $\text{H}_2\text{O}$ , 不过白腐真菌普遍存在降解周期长、对环境耐受性差等特点<sup>[10-12]</sup>。近年来,研究者不断发掘新的木质素降解菌,包括真菌和细菌。

### 1.1 木质素降解真菌

目前研究最为广泛的木质素降解菌大部分属于真菌<sup>[13-15]</sup>。研究表明,一些真菌分泌的木质素降解酶种类多,且可利用菌丝有效破坏植物细胞壁结构,为其分泌的多种木质素胞外氧化酶的渗透提供通道<sup>[12]</sup>。根据促使木材腐烂后形成腐朽的颜色或硬度区分,木质素降解真菌可以分为白腐真菌、褐腐真菌(brown-rot fungi)和软腐真菌(soft-rot fungi),其中白腐真菌降解木质素的能力相对较强<sup>[16-17]</sup>。白腐真菌分泌的胞外氧化酶主要

是漆酶和过氧化物酶,可以同时导致木质素内部醚键和 C-C 键的断裂及侧链的去除,对木质素降解率高<sup>[12]</sup>。例如 Chen 等<sup>[18]</sup>以玉米秸秆作为平菇(*Pleurotus ostreatus*)的发酵基质,30 d 后玉米秸秆木质素含量从 18.45% 下降到 8.35%,降解率达 54.7%。且通过扫描电子显微镜(scanning electron microscope, SEM)观察,发酵 10 d 后平菇菌丝体覆盖了玉米秸秆整个表面,细胞壁被大面积破坏。Sharari 等<sup>[19]</sup>利用黄孢原毛平革菌(*Phanerochaete chrysosporium*)降解甘蔗木质素的研究结果表明,发酵 5 d 后对甘蔗木质素降解率达到 70%。根据胞外氧化酶作用对象不同,白腐真菌对木质素的降解有选择性和非选择性 2 种途径。选择性白腐真菌如虫拟蜡菌(*Ceriporiopsis subvermispora*)、污叉丝孔菌(*Dichomitus squalens*)和黄孢原毛平革菌等菌株分泌的胞外氧化酶主要作用于木质素<sup>[20]</sup>。非选择性白腐真菌如云芝(*Trametes versicolor*)和木

蹄层孔菌(*Fomes fomentarius*)等菌株不仅可降解木质素, 还可降解纤维素和半纤维素<sup>[21-22]</sup>。褐腐真菌一般可有效降解纤维素和半纤维素。但有研究表明部分褐腐真菌不仅可通过芬顿氧化(Fenton-oxidation)产生的羟基自由基(-OH)氧化和降解木质素, 也能产生胞外氧化酶非选择性氧化木质素单体与侧链之间的化学键<sup>[23-24]</sup>。Knežević等<sup>[14]</sup>利用小麦秸秆为唯一碳源培养松生拟层孔菌(*Fomitopsis pinicola*)的研究表明, 发酵 10 d 后, 锰过氧化物酶检测到最大活性(3 235 U/L), 漆酶活性微弱, 14 d 后木质素的降解率达到 32%。软腐真菌同样可以有效降解纤维素和半纤维素。有研究表明部分软腐真菌可以通过氧化紫丁香基单体来降解木质素<sup>[11]</sup>, 但 Hamed<sup>[25]</sup>证明黑曲霉菌(*Aspergillus niger*)和产黄青霉菌(*Penicillium chrysogenum*)等软腐真菌对木质素只有修饰功能, 无降解作用。虽然已知的褐腐真菌和软腐真菌对木质素的降解能力不如白腐真菌, 但探究其对木质素的作用机制有利于更加全面地阐明微生物对木质素的降解机理。

## 1.2 木质素降解细菌

随着多组学技术的发展, 通过对木质素降解细菌的不断挖掘, 已从不同的栖息地(如土壤、腐烂的木材、废水处理厂和动物肠道)中鉴定出具有木质素降解能力的细菌(表 1)<sup>[14,26-33]</sup>。目前, 木质素降解细菌的研究主要集中在链霉菌属(*Streptomyces*)等几个细菌属中<sup>[34]</sup>。在链霉菌属中, 对于 *Streptomyces viridosporus* T7A 降解木质素的研究较为丰富。Zeng 等以小麦秸秆为底物对 *S. viridosporus* T7A 培养 3 周后发现, 麦秸木质素含量由 17.65% 下降至 14.71%, 研究表明, 该菌株产生的包括漆酶和过氧化物酶在内的多种木质素氧化酶可将木质素降解为水溶性聚合物, 对木质素结构中的羰基(-C=O)和甲氧基(CH<sub>3</sub>O-)的修饰也很显著<sup>[26]</sup>。另外, Maria 等<sup>[35]</sup>从热带河口生态系统中分离出 5 株链霉菌, 其中 *S. albogriseolus* 表现出漆酶和木质素过氧化物酶活性, 在培养 10 d 后对木质素的降解率达到 40%。除链霉菌属外, 红球菌属(*Rhodococcus*)的一些菌株也表现出较高的木质素降解活性。研究表明, *R. opacus* 和 *R. jostii* 在降解木质

表 1 已报道的部分可降解秸秆木质素的真菌和细菌

Table 1 Partial fungi and bacteria that have been reported to degrade straw lignin

Type	Strains	Straw substrate	Source	Reference
Fungi	<i>Lenzites betulina</i>	Corn	-	[13]
	<i>C. versicolor</i>	Corn	-	[13]
	<i>D. squalens</i>	Wheat	-	[14]
	<i>Fomitopsis pinicola</i>	Wheat	-	[14]
	<i>P. chrysosporium</i>	Corn, rice	-	[15]
	<i>Lentinula edodes</i>	Corn, rice	-	[15]
	Bacterium	<i>S. viridosporus</i>	Wheat, corn	Soil
<i>R. jostii</i>		Wheat	Soil	[28-29]
<i>R. erythropolis</i>		Wheat	Wood, soil	[30]
<i>B. amyloliquefaciens</i>		Cigarettes and tobacco	Soil	[31]
<i>Pseudomonas</i> sp.		Wheat	Termite gut	[32]
<i>Ochrobactrum pseudintermedium</i>		Corn, rice, peanut vine	Water buffalo rumen	[33]
<i>B. sonorensis</i>				
<i>Klebsiella pneumoniae</i>				

素过程中会产生苯酚等芳香族化合物,而这些化合物最终会被红球菌吸收利用,进而提高对木质素的降解活性<sup>[36-37]</sup>。在芽孢杆菌属(*Bacillus*)中,*B. amyloliquefaciens*<sup>[31]</sup>、*B. flexus*<sup>[38]</sup>和*B. lignophilus*<sup>[39]</sup>,均被报道具有降解木质素的能力,且降解率分别达到28.55%、30.00%和9.10%。

虽然已发掘的真菌相较于细菌对木质素降解效率高,但对极端环境的耐受性较差,在实际应用方面受到较大的局限性<sup>[40-41]</sup>。而细菌除对极端环境具有更强的适应性之外,还具有繁殖快、基因组更小便于分子遗传和蛋白质表达等优势<sup>[42]</sup>。因此,探索构建木质素降解复合菌群,优势互补,可能是大规模降解和利用木质素的突破口。

### 1.3 复合菌群

由于组成木质素的分子结构复杂且含有多种芳烃结构,需要多种降解酶共同作用才能提高降解效率。多项研究结果表明,复合菌群对木质素的降解效率要优于单一菌株<sup>[43-45]</sup>。例如Cui等<sup>[13]</sup>将桦革褶菌和云芝联合培养组成复合菌群,在2种白腐真菌的协同作用下,漆酶和锰过氧化物酶的活性显著增强,最大酶活分别为18.06 U/mL和13.58 U/mL,在相同培养条件下,与桦革褶菌和云芝单一培养相比,从该复合菌群中获得的漆酶和锰过氧化物酶活性均提高了40%,且木质素降解率为50%,高于桦革褶菌(26.6%)和云芝(37.2%)单一菌株木质素降解率。与之研究结果类似的是,Jiménez-Barrera等<sup>[44]</sup>将血红密孔菌(*Pycnoporus sanguineus*)和布氏白僵菌(*Beauveria brongniartii*)共培养后,漆酶和锰过氧化物酶的活性分别增强了6倍和2.3倍。研究表明,2种木质素降解细菌联合培养同样能有效提高木质素降解率。Yadav等<sup>[43]</sup>从污泥中筛选得到的枯草芽孢杆菌(*B. subtilis*)和肺炎克雷伯菌对牛皮纸木质素的降解率分别为39%和44%,而枯草芽孢杆菌和肺炎克雷伯菌在相同条件下联

合培养后,对牛皮纸木质素的降解率达到85%。真菌与细菌通过联合培养对木质素降解同样可以起到促进作用。Iimura等<sup>[45]</sup>从腐朽木材中发现了与白腐真菌共存的5种甲基杆菌属(*Methylobacterium*)细菌,甲基杆菌可以将白腐真菌降解木质素产生的甲醇转化为甲醛,此前报道了甲醛可以阻止木质素解聚产生的小分子化合物缩合反应<sup>[46]</sup>,该研究表明甲基杆菌通过与白腐真菌相互协作能间接提高木质素降解率。Kamei<sup>[47]</sup>通过将云芝与在腐朽木材中分离得到的贪铜菌属(*Cupriavidus*)细菌和肠杆菌属(*Enterobacter*)细菌分别联合培养15 d后研究结果表明,漆酶活性显著增强,木质素降解率均提高5%左右。多项研究表明,复合菌群的组建可以克服单一菌株降解木质素效率低的问题,整合多种木质素代谢途径,形成更加全面的木质素代谢网络,有利于提高木质素降解效率。

## 2 微生物降解木质素的酶系组成

微生物降解木质素是一个由微生物分泌的胞外氧化酶引发木质素内部化学键断裂的过程。越来越多的木质素降解酶被挖掘并应用于木质素的解聚和矿化中。其中,漆酶和过氧化物酶在具有木质素降解作用的微生物中发现较多,作用机制也得到了论证。

### 2.1 漆酶

漆酶(laccase, Lac)是一种蓝色多铜氧化酶,大多数已知的漆酶通常具有1个单核位点( $T_1Cu$ )和1个三核位点( $T_2Cu$ 和 $T_3Cu$ )<sup>[48]</sup>。每个漆酶单体含有4个铜原子, $T_1$ 和 $T_2$ 位点各包含1个铜原子,而 $T_3$ 位点包含2个铜原子<sup>[49]</sup>。漆酶利用氧气作为电子受体通过3个步骤来氧化木质素底物:(1)通过接受来自底物的电子并产生自由基来还原 $T_1$ 位点的铜原子;(2)电子从 $T_1$ 转移到 $T_2/T_3$ 三核位点;(3)在 $T_2/T_3$ 三核位点中

将分子氧还原为水<sup>[50-51]</sup>。漆酶氧化还原电位较低, 只能氧化酚类化合物, 但当有 2,2-联氮-二(3-乙基-苯并噻唑-6-磺酸)二铵盐 [2,2-Biazo-bis(3-ethyl-benzothiazole-6-sulfonic acid) diammonium salt, ABTS]或 1-羟基苯并三唑 (1-hydroxybenzotriazole, HBT)等小分子介质存在并充当载体的情况下, 漆酶可以催化非酚类化合物的氧化, 提高木质素降解效率<sup>[52]</sup>。Gutiérrez等<sup>[53]</sup>在利用长绒毛栓菌(*Trametes villosa*)分泌的漆酶与 HBT 小分子介质结合分别降解桉树和狼尾草木质素的研究中发现, 当漆酶单独处理 2 种底物时, 木质素降解率均小于 5%, HBT 小分子充当介质后木质素降解率分别提高到 48%和 32%。虽然化学介质能增强漆酶对木质素的降解能力, 但其成本过高阻碍了在实际生产中的应用。与此方法相比, 高产漆酶菌株的筛选以及发酵培养基条件的优化仍然是当前的研究热点。Li等<sup>[54]</sup>在腐朽木材中分离得到栓菌属(*Trametes*)真菌, 命名为 *Trametes* sp. LS-10C, 在调整最适 pH 值为 4.0, 最适温度为 40 °C 后研究发现, 在 10 L 发酵罐中发酵 9 d 后漆酶产量达到最大值 873.82 U/mL。漆酶广泛存在于木质素降解微生物中, 表 2 列举了分泌漆酶的部分菌株以及最适产酶条件<sup>[55-60]</sup>。与细菌漆酶相比, 大部分真菌漆酶的氧化还原电位较高, 可以氧化具有高氧化还原电位的化合物<sup>[61]</sup>, 而对于影响漆酶的产生和活性的环境因素(如温度、酸碱度等), 细菌漆酶则表现出更强的耐受性和适应性。

## 2.2 过氧化物酶

目前研究发现的参与木质素降解过程的过氧化物酶主要包括木质素过氧化物酶(lignin peroxidase, LiP)、锰过氧化物酶(manganese peroxidase, MnP)、多功能过氧化物酶(versatile peroxidase, VP)和染料脱色过氧化物酶(dye decolorization peroxidase, DyP)<sup>[62]</sup>。

LiP 以 H<sub>2</sub>O<sub>2</sub> 作为氧化剂, 并且需要 3,4-二甲氧基苯醇(藜芦醇)作为电子供体和辅助因子来完成氧化反应<sup>[63-64]</sup>。LiP 具有较高的氧化还原电位, 具有独自催化非酚类芳香族底物中 C-C 键和醚键氧化的能力<sup>[65]</sup>。MnP 主要催化酚类木质素化合物中 C-C 键的裂解<sup>[66-67]</sup>, 但研究表明, 添加如藜芦醇等酚类中间体或不饱和脂肪酸可以使 MnP 有效氧化非酚类化合物, 且在培养过程中, 向培养基中添加 Mn<sup>2+</sup>或 Cu<sup>2+</sup>能明显刺激 MnP 的产生, 并增强 MnP 的木质素降解活性<sup>[66-68]</sup>。VP 广泛存在于黑管菌属(*Bjerkandera*)和侧耳菌属(*Pleurotus*)中<sup>[10]</sup>。VP 是一种双功能酶, 既可以氧化藜芦醇、甲氧基苯和非酚类木质素化合物, 还可以氧化各种具有高或低氧化还原电位的芳香族底物<sup>[69]</sup>。因此, VP 除用于木质素降解外, 还可用于生物质脱木质素<sup>[70]</sup>。DyP 的序列和结构与其他过氧化物酶不同, 根据序列特征, DyPs 可分为 4 类, A、B 和 C 型 DyP 广泛存在于细菌中, D 型 DyP 则主要由真菌产生<sup>[71]</sup>。4 类 DyP 均具有过氧化物酶活性和特征, 其中 C 和 D 型 DyP 对木质素底物氧化活性较高<sup>[72]</sup>。表 2 列举了产过氧化物酶的部分菌株以及最适产酶条件, 以供参考<sup>[60,73-79]</sup>。

除上述介绍的漆酶和过氧化物酶以外, 直接参与木质素解聚和矿化的酶还包括  $\beta$ -醚酶、脱氢酶、脱羧酶、单加氧酶、双加氧酶、*O*-去甲基化酶和甲基转移酶等。由于酶的催化底物专一性, 每种木质素降解酶仅作用于特定的结构或化学键, 因此木质素的完全降解需要多种酶的协同作用。但由于不同酶间影响酶活性的环境及物质因子不同, 进而将不同的木质素降解酶整合到同一个稳定的协作系统中成为一个难题, 导致木质素降解效率仍然很低。因此参与木质素代谢的完整酶系、分泌机制、酶间互作机制以及影响酶活性的各种因素等是下一步研究的重点方向。

表 2 部分菌株中的漆酶和过氧化物酶及最适产酶条件

Table 2 Laccase and peroxidase in some strains and the optimal conditions for enzyme production

Type	Strains	Optimum pH	Optimum temperature (°C)	Reference
Laccase	<i>P. ostreatus</i>	3.0	50	[55]
	<i>Ganoderma lucidum</i>	3.0	60	[56]
	<i>T. trogii</i>	4.0	60	[57]
	<i>Cerrena unicolor</i>	2.6	45	[58]
	<i>Clostridium botulinum</i>	7.0	50	[59]
	<i>K. pneumoniae</i>	5.0	50	[60]
Lignin peroxidase	<i>P. chrysosporium</i>	3.0	40	[73]
	<i>Acinetobacter</i> sp.	2.0	60	[74]
Manganese peroxidase	<i>G. lucidum</i>	4.5	25	[75]
	<i>K. pneumoniae</i>	5.0	50	[60]
Versatile peroxidase	<i>Pleurotus eryngii</i>	3.0	45	[76]
	<i>Physisporinus vitreus</i>	5.0	40	[77]
Dye decolorization peroxidase	<i>P. sapidus</i>	4.5	15–30	[78]
	<i>Dictyostelium discoideum</i>	4.0	20–40	[79]

### 3 微生物介导的木质素降解机制及衍生芳烃的代谢路径

#### 3.1 降解机制

目前,针对微生物介导的木质素降解机制的研究虽不完善,但一般降解过程已经构建出来。研究表明,微生物分泌的胞外氧化酶(如漆酶和过氧化物酶)首先引发木质素内部的 C-C 键、C-O 键断裂等裂解反应并产生苯氧基自由基中间体,将木质素解聚成简单的单体形式,如对香豆醇、松柏醇和芥子醇<sup>[80]</sup>。随后,木质素解聚产生的芳香族异质混合物通过细菌独特的“生物漏斗”途径被各种细胞内酶(如双加氧酶、脱羧酶、甲基转移酶和谷胱甘肽转移酶等)代谢转化为中心中间体——丁香酸、香草酸和对羟基苯甲酸,最终中间体被多种环裂解途径进一步降解进入三羧酸循环(tricarboxylic acid cycle, TCA)<sup>[27,81-83]</sup>(图 2)。有研究表明,在木质素降解初期,胞外氧化酶分子较大而无法穿透细胞壁,因此低分子量化合物(植物细胞外存在的芳香族化合物如黄酮、单宁和木酚素等)便作为可扩散的氧化剂和

酶系统的“电子穿梭器”(electron shuttles)直接与木质素反应,引发一系列木质素内部化学键的裂解,导致木质素的解聚<sup>[84-85]</sup>。由此可见,在微生物降解木质素过程中,除酶发挥主导作用外,各种辅助因子也扮演重要角色。进一步研究当中,除要重点挖掘参与木质素代谢的各种酶系外,还可利用组学等技术手段,充分挖掘与木质素代谢相关的各种辅助因子及其作用机制,以更加全面地阐明木质素的微生物降解机制。

#### 3.2 “生物漏斗”式木质素衍生芳烃的降解

研究表明,细菌在木质素衍生的非均相芳香族化合物的矿化过程中起着主导作用。为克服木质素的顽固性,自然界中一些木质素降解细菌进化出“生物漏斗”式木质素衍生芳烃的代谢途径(图 2B):即木质素降解细菌会通过  $\beta$ -芳基醚和联苯等多种代谢途径,将木质素解聚衍生的芳香族化合物转化为丁香酸、香草酸和对羟基苯甲酸中间体,随后便通过 O-去甲基化等反应将中间体转化为儿茶酚等衍生物,最终衍生物的苯环被多种环裂解途径破坏进入三羧酸循环(TCA),从而实现细菌对木质素衍生芳烃的降解



和利用<sup>[27,83,86]</sup>。“生物漏斗”式木质素衍生芳烃降解方式的提出,为解决木质素芳香族异质混合物难降解的问题提供可能。如恶臭假单胞菌 KT2440 (*Pseudomonas putida* KT2440)分泌的双加氧酶可以将儿茶酚衍生物转化为顺,顺-己二烯二酸<sup>[87]</sup>, 短小芽胞杆菌 ZB1 (*B. pumilus* ZB1)分泌的单加氧酶将丁香酚和异丁香酚转化为香兰素<sup>[88]</sup>, 少动鞘氨醇单胞菌 SYK-6 (*Sphingomonas paucimobilis* SYK-6)则可以通过  $\beta$ -芳基醚和香兰豆素等多种代谢途径降解木质素芳香族化合物<sup>[86]</sup>。同时,如图 2 所示<sup>[27,83]</sup>,该衍生芳烃的代谢方式与上游木质素解聚相结合,降解细菌可以迅速代谢木质素解聚衍生的芳烃混合物,阻断木质素解聚后再聚合的可能,为木质素的降解转化提供可行性的方案,具有应用前景。目前已经报道具有降解木质素衍生芳烃能力的细菌主要集

中在少数菌株中,需进一步挖掘具有代谢木质素衍生芳烃能力的菌株。此外,该方案证明组建木质素降解复合菌群,尤其是真菌与细菌进行合理配伍降解木质素的可行性,这将是下一步的研究重点。

## 4 木质素降解微生物在秸秆肥料化技术中的应用进展

### 4.1 提高秸秆腐熟效率

如前述,木质素是微生物降解秸秆木质纤维素的限速因子,同时也会降低秸秆腐熟的实际效率。一些高效降解秸秆木质素的微生物能有效地提高秸秆降解腐熟效率。Saha 等<sup>[89]</sup>以 26 株白腐真菌为研究对象,玉米秸秆为底物,在相同条件下培养 30 d 后发现黄孢原毛平革菌 NRRL-6370 (*P. chrysosporium* NRRL-6370)对玉米秸秆中木质

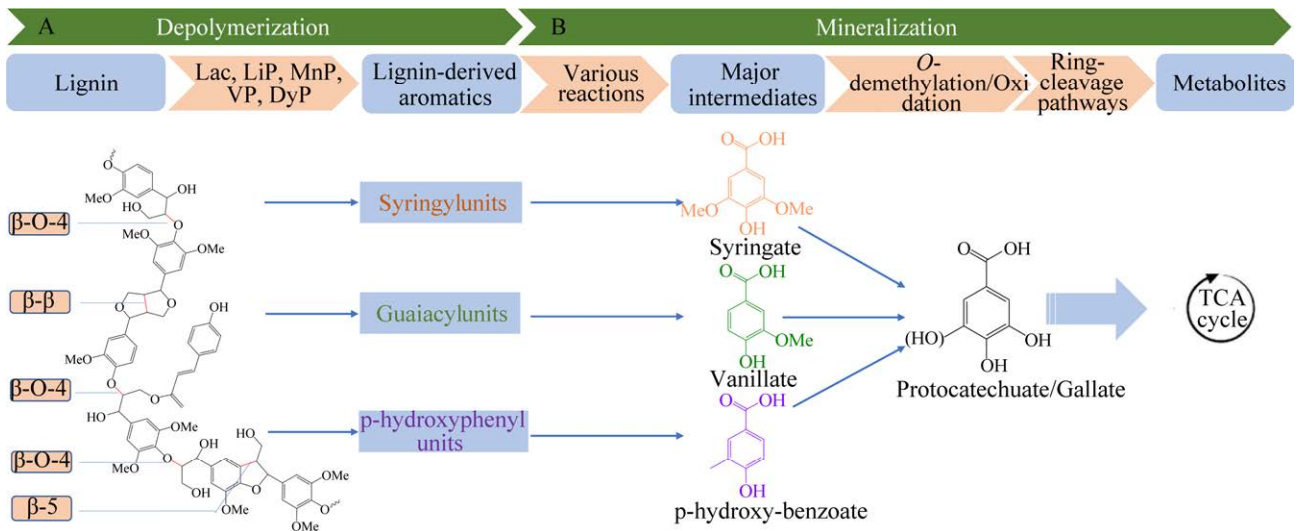


图 2 微生物对木质素的解聚及衍生芳烃代谢机制示意图<sup>[27,83]</sup>

Figure 2 Schematic diagram of microbial depolymerization of lignin and metabolism of derived aromatic hydrocarbons<sup>[27,83]</sup>. Microbial degradation of lignin can be divided into two stages: Depolymerization and mineralization. A: The depolymerization stage of lignin. The C–C bonds and C–O bonds inside lignin are first oxidized by laccase and peroxidase secreted by microorganisms, and the lignin macromolecules are depolymerized into simple monomer forms. B: The mineralization stage of lignin. The aromatic compounds produced by lignin depolymerization are converted into intermediates through various metabolic pathways, and finally the intermediates are further degraded by various ring cleavage pathways and enter the tricarboxylic acid cycle.



素的降解率最大(51%), 血红密孔菌 NRRL-FP-103506-Sp (*Py. sanguineus* NRRL-FP-103506-Sp) 次之(46.7%), 而木质素的降解会增加底物的孔径, 从而为纤维素酶和半纤维素酶提供一个更容易接触目标的表面积, 使得纤维素和半纤维素更容易降解, 最终提高玉米秸秆腐熟效率。与此研究结果类似的是, Song 等<sup>[90]</sup>将烟曲霉菌 CLL (*Aspergillus fumigatus* CLL) 接种到含有玉米秸秆的培养基内接续培养, 通过扫描电子显微镜观察到 10 d 后玉米秸秆的表面结构被彻底破坏, 纤维素和半纤维素完全暴露在可视范围内, 有效提高纤维素酶和半纤维素酶的可及性, 加速秸秆的腐熟效率。2 项研究均有效地阐明利用木质素降解微生物对秸秆进行预处理提高秸秆整体酶解腐熟效率的可行性。

#### 4.2 在秸秆好氧堆肥中的应用

好氧堆肥是一种微生物介导的需氧、嗜热的固态发酵过程, 该过程可以将秸秆转化成作物生长所需要的腐殖质(humus), 是实现秸秆资源化和减量化的高效途径之一。研究表明, 秸秆木质素降解过程中释放的化合物可以作为腐殖质形成的前体, 提高腐殖质的形成效率<sup>[91]</sup>。基于这 2 点原因, 前人有报道接种木质素降解菌有助于堆肥效率的提高。例如, Mei 等<sup>[31]</sup>在研究接种木质素降解菌解淀粉芽孢杆菌 SL-7 对烟草秸秆堆肥腐熟效率影响实验中表明, 堆肥结束时, 接种与未接种堆肥物料相比, 木质素降解率提高 22.26%, 腐殖质的含量提高 3.56 g/kg。并且该研究通过检测对比接种解淀粉芽孢杆菌 SL-7 前后堆肥材料的 C/N 及种子萌发指数(germination index, GI)判断, 接种该菌株有利于秸秆的腐熟, 且能提高堆肥肥效及稳定性。Chen 等<sup>[92]</sup>在玉米秸秆和油菜残渣的堆肥冷却期接种黄孢原毛平革菌的研究结果表明, 接种后与未接种相比, 木质素含量降低 64.3%, 纤维素含量降低 40%, 此外, 腐殖质含量显著增加 55.4%。上述研究结

果皆表明, 接种木质素降解微生物具有克服目前好氧堆肥腐熟效率低, 堆肥产品不均匀的能力。但好氧堆肥过程温度往往起伏较大, 因此, 筛选出对极端环境有着较强抵御作用的极端木质素降解微生物, 可能会对堆肥效率的提高更有帮助。

## 5 研究展望

木质素结构复杂, 且与纤维素、半纤维素组成晶体结构, 是环境微生物降解秸秆的主要障碍。近年来利用高通量测序技术和多组学技术的优势, 对木质素降解菌及其降解机制的研究取得积极的进展, 但仍存在以下 3 方面的问题有待解决: (1) 缺乏对高效率木质素降解细菌和复合菌群的挖掘。虽然目前已报道的真菌降解木质素在实验室层面得到充分论证, 但实际应用中还是会受到孵育周期长、对极端环境耐受性差等因素的影响。而由于细菌具有对极端环境较强的适应性以及能被基因工程改造等优势, 正逐渐成为当前研究的热点<sup>[42]</sup>。不过目前对木质素降解细菌的发掘未能有新的突破, 因此整合目前现有技术手段挖掘出高效的木质素降解细菌, 可能成为实际应用中大规模降解转化木质素的突破口。此外, 复合菌群的构建可以整合不同菌株的优势, 对木质素降解起着积极作用, 但目前对复合菌群之间互作机制的研究较为匮乏。借助高通量组学技术的优势, 挖掘木质素降解复合菌群, 将会为解决目前存在的木质素降解效率低的问题提供方向。(2) 木质素降解酶系的研究尚不完善。木质素的降解需要多种氧化酶的协同作用, 而当前针对多种酶间以及酶与小分子介质之间互作机制的研究较少。因此, 充分利用分子生物学技术研究参与木质素降解和转化的潜在的关键酶系统、分泌机制和酶间互作机制是当前需要解决的关键问题, 这将有助于进一步阐明木质素降解机制。此外, 木质素降解酶针对特定底物发挥作用

的最适环境条件还需进一步研究<sup>[93]</sup>。(3) 木质素降解菌应用于秸秆还田方面的报道较少。当前我国对可收集秸秆超一半以上作还田处理<sup>[1]</sup>, 秸秆依靠土壤微生物自然降解效率缓慢, 长期存在不利于土壤与大气环境的物质交换, 易导致土壤处于缺氧环境, 不利于作物生长<sup>[52]</sup>。因此, 提高秸秆还田降解效率是当前亟需解决的问题, 但目前木质素降解菌在秸秆还田中应用的研究较少。利用多种底物筛选方法, 目前课题组已从不同生境(来自土壤、基质、玉米秸秆、堆肥、药渣等)中分离获得具有显著木质纤维素降解能力的真菌和细菌<sup>[94]</sup>。其中通过解析非洲哈茨木霉(*Trichoderma afroharzianum*)基因组和生物特性研究表明, 非洲哈茨木霉不仅可以产生纤维素酶等水解酶通过水解真菌病原菌细胞壁达到抑制病原菌的效果, 还能分泌木质素降解酶系, 在秸秆还田中具有应用前景<sup>[95-96]</sup>。下一步, 将对秸秆还田之后秸秆降解菌群演化规律做进一步研究, 并结合筛选出适用于还田的木质素降解菌及复合菌群。

综上所述, 微生物介导的木质素降解是需要多种酶系统通过多种代谢途径实现的复杂过程。而挖掘新的高效木质素降解微生物和降解酶, 并深层次地解析木质素降解转化机制将为实现农作物秸秆的资源化、减量化和无害化提供理论基础。

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