



极端微生物降解木质素的研究进展

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摘 要: 木质素是自然界最丰富的芳香族生物聚合物资源, 由于其复杂性和高度聚合的芳香族结构, 木质素很难被普通微生物降解。来自极端环境的木质素降解微生物被认为是木质素生物加工的合适候选者。本文对几类能够降解木质素的极端微生物及其产生的极端酶进行了归纳总结, 并阐明了极端酶的性质、催化机制及其代谢途径。讨论了通过多组学方法鉴定新型极端微生物和极端酶的前景, 并对极端微生物的开发和利用方法进行了展望, 以期为后续筛选和开发更加高效的木质素降解菌株提供参考。

关键词: 木质素; 极端微生物; 极端酶; 生物降解; 组学技术

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Lignin degradation by extremophiles

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Abstract: Lignin, the most abundant aromatic biopolymer resource in the nature, is difficult to be degraded by common microorganisms due to its complexity and highly aggregated aromatic structure. Lignin-degrading microorganisms from extreme environments are considered as suitable candidates for lignin bioprocessing. This review summarizes several types of extremophiles capable of degrading lignin and the extremozymes produced by them and elucidates the properties, catalytic mechanisms, and metabolic pathways of the extremozymes. Furthermore, this article discusses the prospects for the identification of novel extremophiles and extremozymes by multi-omics and makes an outlook on the development and utilization methods of extremophiles, with a view to providing a reference for the subsequent screening and development of more efficient lignin-degrading strains.

Keywords: lignin; extremophiles; extremozymes; biodegradation; omics

木质纤维素生物质是地球上最丰富的自然资源, 其中木质素是主要成分之一, 是芳香族聚合物的重要资源^[1]。由于木质素结构的异质性和复杂性, 在其解聚和降解的过程中, 往往需要涉及与物理、化学和生物相关的多种方法, 如电子束辐照改善木质素的降解性能^[2]、使用离子液体改性木质素^[3]、利用漆酶(laccase, Lac)、木质素过氧化物酶(lignin peroxidase, LiP)、锰过氧化物酶(manganese peroxidase, Mnp)、染料脱色过氧化物酶(dye decolorization peroxidase, DyP)等进行酶促降解^[4]。木质素是一种普遍存在的碳能源, 寻找合适的方法(尤其是绿色环保且依赖于自然界微生物的方法)降解木质素以增强其应用价值是非常有意义的。极端微生物是最适合生活在极端环境中的微生物的总称, 根据其生存极端环境的不同, 可分为嗜冷微生物、嗜热微生物、嗜酸微生物、嗜碱微生物、嗜盐微生物、嗜旱微生物、嗜压微生物等, 特殊的生存

条件导致其具有特殊的遗传背景和代谢途径, 并可产生功能特殊的酶类和活性物质^[5]。

在木质纤维素的应用中, 木质素的降解十分关键。在农业领域, 全球每年都会产生大量的含木质素的固体废弃物, 如秸秆、甘蔗渣、糠壳^[6], 而只有少数得到了有效的再利用, 大部分被直接丢弃或焚烧^[7], 尤其在寒冷地区, 这些含木质素废物的降解和转化效率更低^[8]。在畜牧业中, 植物性饲料中木质素的存在降低了动物饲料的消化利用率, 影响畜牧业的健康和快速发展^[9-10]。在工业领域, 由于木质素难以降解, 阻碍了生物质资源转化为生物燃料、生物塑料、生物肥料和其他增值化学品^[11]。因此, 木质素的高效降解是控制环境污染、提高畜牧业饲料利用率和经济效益、实现木质素资源化利用的有效途径, 而开发能够耐受木质素生物精炼过程中的高温、低温、高渗透压、强酸、强碱条件的酶或微生物至关重要。极端微生物

已经进化出许多与代谢活动、生存和保护策略有关的适应机制,使其能够适应高海拔地区、深海、热液、高盐度和极地等极端环境并大量繁殖,产生不同种类的活性酶。由于其独特的生理特性和酶学特征,极端微生物和极端酶从生产医疗和工业用途的生物分子到对废水和沉积物中的有毒污染物进行生物修复均具有潜在的应用价值^[12-14]。本文综述了极端微生物降解木质素的策略,介绍了降解木质素的极端酶及其降解机制,讨论了通过多组学方法鉴定新型极端微生物和极端酶的前景,并对极端微生物的开发和利用方法进行了展望。如果能够获得高效降解木质素的极端微生物,并深入了解其降解机制,就能够在极端的生物过程中降解/发酵木质素,促进该生物资源的利用。

1 木质素的结构和组成

木质素是一种异质性多酚材料,主要由 3 种肉桂醇前体(单木质素)组成:芥子醇(3,5-dimethoxy-4-hydroxyphenylpropane, S unit)、松柏醇(4-hydroxy-3-methoxyphenylpropane, G unit)和对香豆醇(4-hydroxyphenylpropane, H unit) (图 1A)^[1]。天然木质素的组成和结构随其物种的不同而变化,并表现出局部和季节的变化。天然木质素通常可分为 3 种类型:硬木(被子植物)、软木(裸子植物)和草本(禾本科)木质素。硬木木质素主要由松柏醇和芥子醇前体组成,软木木质素主要由松柏醇前体组成,草本木质素具有 3 种类型的前体^[15]。表 1 显示了不同木质纤维素生物质中木质素和 3 种肉桂醇前体的含量^[1,15]。

在生物聚合(木质化)过程中,这些木质素单体通过氧化缩合连接在一起,形成多种键^[16] (图 1B)。自然界中的木质素聚合不受酶或蛋白质控制,一旦产生自由基,就是一个纯粹的化学过程,可分为 2 个阶段:在第一阶段,酚类

木质素单体自由基二聚形成 β -O-4、 β - β 和 β -5 脱氢二聚体,这是木质素聚合物中最常见的键;随着聚合的进行,低聚酚末端单元通过 5-5 和 4-O-5 型键连接;同时,在木质素单体和预先形成的 β -芳基醚二聚末端单元之间形成 β -1 型连接;这种自由基偶联过程在木质素结构中缺乏规则和有序的重复单元(图 1C),因此木质素的利用受到限制^[1,17]。

2 降解木质素的极端微生物

生物转化和降解是利用木质素的最重要机制之一。目前已知许多真菌和细菌均具有优异的木质素降解能力,它们对木质素的分解和代谢具有多种不同策略,并且不产生二次污染,绿色环保。研究表明热红短芽孢杆菌(*Brevibacillus thermoruber*)^[18]、褐色喜热裂孢菌(*Thermobifida fusca*)^[19]、热解纤维梭菌(*Clostridium thermocellum*)^[20]、解硫酸素芽孢杆菌(*Aneurinibacillus* sp.) LD3^[21]、节杆菌(*Arthrobacter* sp.) C2^[22]等极端微生物均具有降解木质素的作用。近年来,许多研究倾向于筛选并构建出单一高效的木质素降解菌株或构建人工复合菌系协同降解木质素,以提高其利用价值。

2.1 单一菌株降解木质素

研究表明,木质素在碱性溶液中的溶解度随所用溶剂的极性、溶液黏度和所用碱性试剂的阳离子半径而产生较大变化,高 pH 值不仅有助于提高木质素的溶解度,而且还能降低木质素粒径^[23]。为了在碱性溶液中实现木质素生物转化,Xu 等^[24]在 pH 11.0 的碱性条件下筛选出了几种耐碱木质素分解细菌,其中萨克利夫氏菌(*Sutcliffiella* sp.) NC1 被证明是一种嗜碱细菌,其木质素过氧化物酶在碱性条件下表现出高度活性。Jiang 等^[22]为解决中国寒冷地区大部分富含木质纤维素的农业剩余物降解受限的问

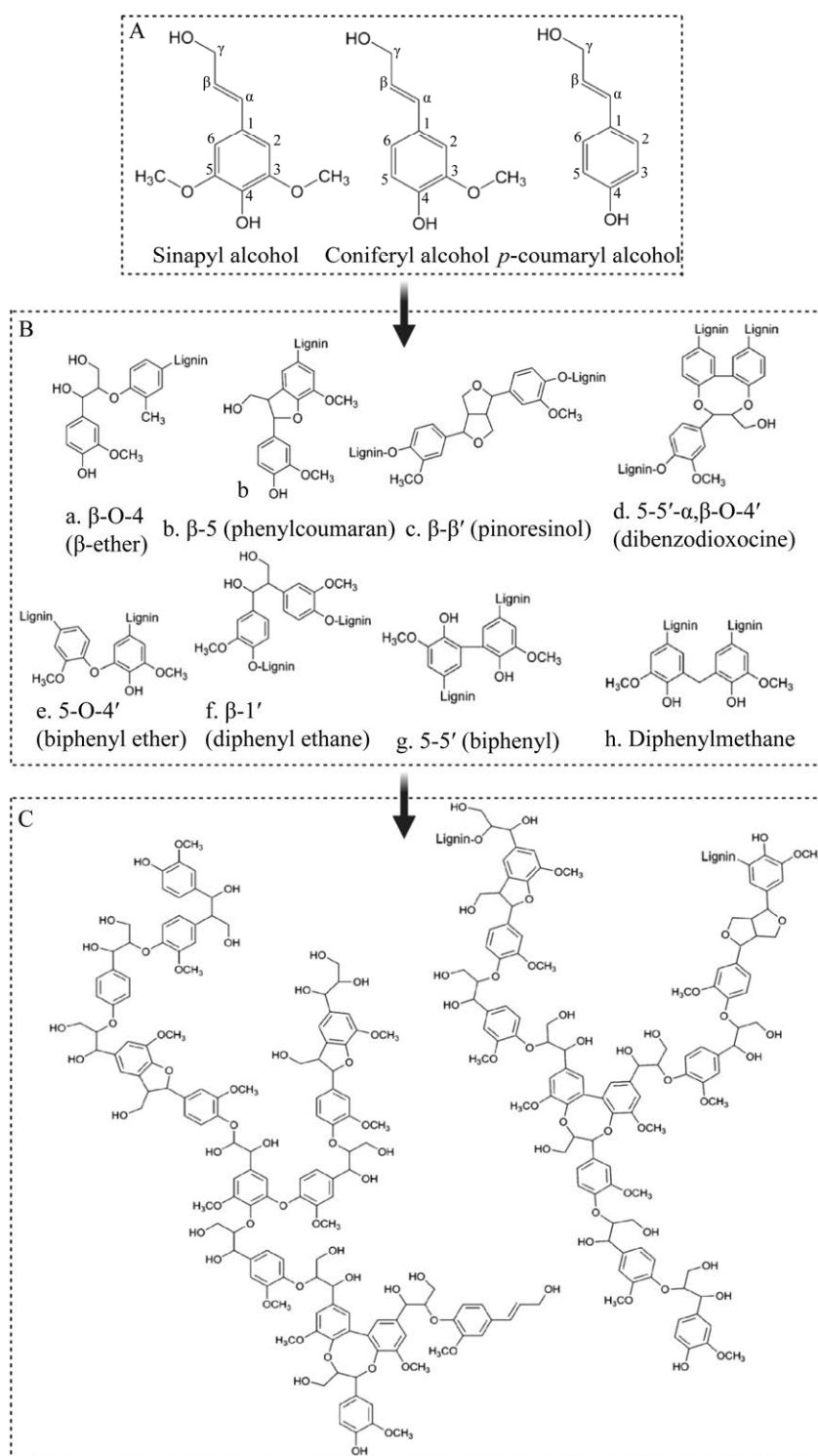


图 1 木质素结构特征 A: 木质素中的主要肉桂醇前体^[1]. B: 木质素单体之间不同类型的键合^[16]. C: 可能的木质素结构^[17]

Figure 1 Lignin structural features. A: Main cinnamyl alcohol precursors in lignin^[1]. B: Different types of bonding between lignin monomers^[16]. C: A possible lignin structure^[17].

表 1 不同木质纤维素生物质中木质素和肉桂醇前体的含量^[1,15]

Table 1 The contents of lignin and cinnamyl alcohol precursors in different lignocellulosic biomass^[1,15]

Species	Lignin (%)	Cinnamyl alcohol precursor (%)		
		<i>p</i> -coumaryl alcohol	Coniferyl alcohol	Sinapyl alcohol
Hardwood	25–38	0–8	25–50	45–75
Softwood	20–30	<5	>95	0
Herbaceous plant	8–15	5–35	35–80	20–55

题, 从−10 °C的冻土中分离出一株耐冷细菌节杆菌 C2, 该菌株产酶活性的最佳条件为初始 pH 6.7、温度 14.9 °C。研究发现耐冷菌株 C2 可应用于造纸黑液的处理, 该菌株具有良好的冷适应性和木质素降解能力, 在 15 °C 时, 3 g/L 的木质素溶液的降解率为 65.5%, 矿化率为 43.9%^[25]。此外, Wu 等^[21]筛选出了耐热碱木质素降解菌株解硫胺素芽孢杆菌 LD3 并将其应用于餐厨垃圾堆肥过程, 该菌株具有较强的碱木质素降解能力和木质素分解酶活性, 在降解过程中产生许多低分子量化合物, 促进了芳香结构的形成。同时, 由于木质素降解过程中会产生芳香族化合物, 通常对细胞有毒, 所以木质素降解过程需要微生物能够耐受这些芳香族化合物的抑制作用或毒性。因此, 为了克服酚类抑制剂过量的问题, 需要利用能够分泌耐热木质素酶并降解这些酚类化合物的微生物。从深海沉积物中分离得到的耐碱嗜盐细菌嗜木质素芽孢杆菌 (*Bacillus ligniniphilus*) L1 表现出显著的木质素降解能力, 其木质素解聚混合物中含有数十种芳香族化合物^[26]。此外, 韩宇杰等^[27]从森林土壤、秸秆还田土及腐烂的木材等样品中筛选出了 3 株靶向降解木质素的细菌菌株, 在 pH5.0–9.0, 培养温度 10–50 °C 下, 3 株细菌均可以正常生长, 并具有降解木质素的能力。Kang 等^[28–29]在吉林大安国家农田生态系统野外观测研究站玉米田土壤中筛选出一株耐冷类节杆菌 (*Paenarthrobacter* sp.) KN0901, 该菌株在 15 °C 下可同时降解莠去

津和秸秆复合污染物, 并且莠去津与秸秆共同降解使该菌株的生化反应水平有所提高, 进一步消除了莠去津对植物的毒性。综上所述, 使用单一菌株降解木质素时, 可以进行定向筛选, 从而得到适应性更好的微生物, 以提高木质素降解率; 同时, 也可以考虑使用单一菌株对含木质素的废弃物和其他化学污染物共同降解, 这可能会促进微生物的降解作用。

2.2 多种微生物协同降解木质素

由于木质素结构的异质性和生物转化途径的可变性, 单一菌株的木质素解聚效率有限, 高效的微生物反应需要一套复杂的木质素水解酶, 而单一微生物无法产生^[30]。构建微生物共培养体系或自然富集培养细菌群落已被认为是提高木质素分解效率的方法, 微生物联合体可以克服单一菌株降解效率受限的问题, 整合多种微生物的木质素代谢途径, 形成更全面的木质素代谢网络^[31–32]。微生物群落, 特别是嗜极微生物群落的发现, 对于制定实现高效木质素生物降解过程的策略至关重要。例如, Wang 等^[33]富集了一组高温(50 °C)木质素降解菌群, 并优化了木质素降解的培养条件, 结合高通量测序技术, 分析了复合菌群的协同效应, 并将高温木质素降解菌群处理过的稻草用于纤维膜的制备。Yadav 等^[34]从纸浆造纸厂废水污染场地中分离出枯草芽孢杆菌 (*Bacillus subtilis*)、生癌肠杆菌 (*Enterobacter cancerogenus*) 和地衣芽孢杆菌 (*Bacillus licheniformis*), 在共同降解木质

素的过程中能够产生高分子量的群体感应信号分子(N-acyl homoserine lactone, AHL), AHL 的产生导致生物膜的形成, 形成生物膜的菌群木质素降解率比单一培养的枯草芽孢杆菌、瘤肠杆菌和地衣芽孢杆菌高出 34%–39%; 同时, 将联合菌群用于废水处理, 水中的主要有机污染物减少, 从而证实了活性生物转化和木质素的生物降解。为探索低温下加速玉米秸秆分解和回归的有效途径, Cheng 等^[35]以生物炭为载体, 利用 9 株耐冷纤维素、半纤维素和木质素降解菌株, 制备了新型耐冷秸秆降解菌群; 在 10–15 °C 下, 该耐冷菌群处理 50 d 后玉米秸秆最大降解率达到 59.3%, 并增加了土壤中有机质、磷、钾的含量; 同时, 该菌群的添加提高了土壤中微生物群落多样性, 改变了特异性选择, 使节杆菌(*Arthrobacter*)、假单胞菌(*Pseudomonas*)和泛菌(*Pantoea*)成为土壤微生物中的优势菌属, 促进了玉米秸秆的降解。在我们之前的研究中, 通过土壤富集获得了由鞘氨醇杆菌(*Sphingobacterium*)、黄杆菌(*Flavobacterium*)、地杆菌(*Pedobacter*)、节杆菌(*Arthrobacter*)、代夫特菌(*Delftia*)和鞘氨醇单胞菌(*Spingomonas*)组成的耐低温木质纤维素降解菌群, 该菌群在 15 °C 的低温下对废纸和秸秆的降解率达到了 21.8% 和 52.0%^[36]。此外, 能降解莠去津和秸秆复合污染物的 KN0901 可以和另一株红球菌(*Rhodococcus* sp.) KD0901 构成人工微生物群落 KDN0901, 该人工复合群落对不同 pH 和温度条件(分别为 5.0–9.0 和 4–35 °C)表现出高度适应性, 尤其是在低温下可同时降解莠去津和邻苯二甲酸二丁酯^[37]。综上所述, 从各种极端环境中筛选具有显著木质素降解能力的极端微生物, 构建能够协同降解木质素的微生物菌群, 也是提高微生物降解木质素效率的重要途径。

此外, 复合极端微生物群落的降解能力往往不是单一的, 在多种微生物共同作用下, 包括木质纤维素在内的多种固体废弃物均能在一定程度上得到降解和转化^[38] (已报道的能够降解木质素的极端微生物的详细信息见表 2)。

3 降解木质素的极端酶及其降解机制

3.1 Lac、LiP、MnP 和 DyP

Lac (EC: 1.10.3.2) 是一种含有 4 个铜离子的多酚氧化酶, 属于铜蓝氧化酶, 以单体糖蛋白的形式存在, 它存在于蘑菇、细菌和植物以及空气中, 催化酚类和非酚类化合物的氧化, 其中耐热 Lac 是一种优异的木质素降解绿色催化剂, 其副产品无化学污染, 需求广泛^[48]。Khan 等^[49]从高地芽孢杆菌(*Bacillus altitudinis*) SL7 中克隆了具有类似 Lac 活性的 CotA 蛋白编码基因, 并在大肠杆菌中进行了表达, 纯化的蛋白酶 CotA-SL7 在宽泛的温度(30–90 °C)和 pH 值(3.0–10.0)范围内都具有活性, 在 55 °C 和 pH 5.0 时活性最佳, 该研究还证明了 CotA-SL7 能裂解木质素聚合物并打开苯环, 具有较高的催化效率。此外, 从嗜木质素芽孢杆菌(*Bacillus ligniniphilus*) L1 中获得的稳定性 Lac 也表现出优异的木质素降解能力, 碱性木质素和磨碎木质素的降解率分别达到 67.0% 和 80.9%^[50]。

LiP (EC: 1.11.1.14) 是一种带有血红素蛋白的糖基化酶, 使用过氧化氢作为氧受体催化木质素聚合物中存在的不同键的裂解。与 Lac 相比, LiP 由于其高氧化还原电位, 无需介体即可有效氧化酚类和非酚类木质素结构^[51]。LiP 可以氧化木质素的重要键, 例如 β -O-4 和 C-C, 这表明它在木质素降解和相关工业应用方面有巨大的潜力^[52]。Rath 等^[53]从印度的一个生物保护

表 2 已报道的部分可降解木质素的极端微生物

Table 2 Some of the extreme lignin-degrading microorganisms that have been reported

Lignin-degrading strains	Growth characteristics	Source	References
<i>Aneurinibacillus</i> sp. LD3	Degradation of lignin up to 61.28% at 50 °C	Soil samples covered with sawdust	[21]
<i>Bacillus amyloliquefaciens</i> SL-7	The strain can be composted for 13 days at temperatures between 55 °C and 62 °C	Tobacco straw	[39]
<i>Bacillus amyloliquefaciens</i> HZ11	Degradation of lignin up to 46.7% at 50 °C	Fungus chaff, straw and cow dung composted to produce seedling substrate	[40]
<i>Serratia</i> sp. AXJ-M	Strain was able to survive in heat stress environments ranging from 50–80 °C, with 50 °C being the optimal temperature for growth	Soil	[41-42]
<i>Stenotrophomonas maltophilia</i> LS-1	The optimal conditions for enzyme production by the strain were pH 8.0 and temperature 15 °C	Frozen soil	[43]
<i>Arthrobacter</i> sp. C2	The optimal conditions for enzyme production activity of this strain were initial pH 6.7 and temperature 14.9 °C	Frozen soil	[22]
<i>Enterobacter</i> sp. YP-2023-9	The strain can tolerate at least 0.60% nicotine	Cow dung	[44]
<i>Chaetomium</i> sp. CS1	Promoting the release of nicotinic actives from tobacco stover	Bottomless sea sediment	[45]
<i>Sutcliffiella</i> sp. NC1	Alkaliphilic bacteria, which do not survive at pH 6.0, grow normally at pH 8.0–11.0	Soil	[24]
<i>Bacillus ligniniphilus</i> L1	The strain has an optimum growth temperature of 30 °C, an optimum pH of 9.0 and can grow at 0–10% total salt concentration, with an optimum salt concentration of 2% (W/V)	Bottomless sea sediment	[26]
<i>Pseudomonas putida</i> KT2440	This strain is well adapted to salt osmotic stress, and at the same time has remarkable metabolic diversity and withstands difficult redox reactions and operational stresses	Soil	[46-47]

区的土壤样品中分离和筛选出一株产 LiP 的菌株,通过响应面分析发现 LiP 在 pH 10.5、温度 30 °C、底物浓度 0.115 mmol/L 和培养时间 42 h 时具有最大活性。

MnP (EC: 1.11.1.13)是含血红素的过氧化物酶之一,主要由寄生在木材上的白腐菌和担子菌产生,在木质素的初始解聚中发挥重要作用,凭借其较高的氧化还原电位, MnP 能够促进酚类木质素的显著降解,并最终释放二氧化碳^[54]。从白腐真菌(*Irpex lacteus* sp.) CD2 中纯化的耐热 MnP 对金属离子和有机溶剂表现出显著的耐受性,并且在 3.5–6.0 的 pH 范围内高度稳

定,最佳温度为 70 °C^[55]。Zhang 等^[47]研究表明, MnP 通过诱导杨树预水解液中的木质素聚合来提高木质素的去除率,同时保证糖类物质含量不受影响。当 Lac、LiP 和 MnP 联合使用降解碱性木质素时,碱性木质素的降解率最高,但其结构完整性较低^[56]。

DyP (EC: 1.11.1.19)是一类血红素过氧化物酶,普遍存在于细菌、放线菌、真菌、古细菌和后生动物中,其在系统发育、结构和机制上与其他过氧化物酶(LiP 和 MnP)不同^[57]。Yu 等^[58]研究发现,嗜热放线菌(*Saccharomonospora viridis*)可以产生稳定的耐热 DyP,其在较宽的

pH 范围(5.0–10.0)和较高温度(50–80 °C)下均有活性。

传统的木质素酶在较窄的反应条件范围内是合适的催化剂,但在高温和极端 pH 等条件下则表现出较低的稳定性^[59]。因此,极端酶是恶劣条件下木质素降解的关键突破口。然而,截至目前极端酶尚未在木质素生物精炼中大规模应用,阻碍极端酶广泛应用的原因之一,是通过非常规分子生物学技术难以高效分离和培养在异源系统中高表达的极端酶^[60]。在今后的研究中应当致力于开发分离和培养极端酶的生物工具,以实现木质素的高效利用。

3.2 降解机制

如图 2A 所示,木质素衍生的芳香族化合物的降解至少涉及 5 种不同的途径^[61–67]。(1) β -芳基醚降解途径,木质素降解菌株首先将愈创木基甘油- β -愈创木基醚(guaiacylglycerol- β -guaiacyl ether, GGE)转化为 α -(2-甲基苯氧基)- β -羟基丙香草酮[α -(2-methoxyphenoxy)- β -hydroxypropiovanillone, MPHPV],随后 MPHPV 的醚键通过还原裂解产生 β -羟基丙吡香草酮(β -hydroxypropiovanillone, HPV)和愈创木酚。(2) 联苯降解途径,5,5-脱氢二香草酸(5,5-dehydrodivanillate, DDVA)首先通过木质素降解菌株进行 O-去甲基化,产生 2,2',3'-三羟基-3'-甲氧基-5,5'-二羧基联苯(2,2',3-trihydroxy-3'-methoxy-5,5'-dicarboxybiphenyl, OH-DDVA),OH-DDVA 在双加氧酶的作用下降解为 4,11-二羧基-8-羟基-9-甲氧基-2-羟基-6-氧代-6-苯基六-2,4-二烯酸酯(4,11-dicarboxy-8-hydroxy-9-methoxy-2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate, DCHM-HOPDA),而后生成香草酸(vanillate, VAN);VAN 代谢是通过 O-去甲基化生成原儿茶酸(protocatechuate, PCA)来解释的;PCA 的降解取决于 PCA 4,5-裂解途径。在该途径中,PCA 的降解可通过 4,5-裂解途径分为 3 种

不同的代谢途径,在最典型的分支之一中,PCA 最初转化为 4-羧基-2-羟基粘康酸酯-6-半醛(4-carboxy-2-hydroxymuconate-6-semialdehyde, CHMS);CHMS 无需酶促作用即可转化为分子内半缩醛形式,然后氧化为 2-吡喃酮-4,6-二羧酸酯(2-pyrone-4,6-dicarboxylate, PDC);接着 PDC 生成酮和烯醇形式的 4-草酰中康酸酯(4-oxalomesaconate, OMA),最终生成 4-羧基-4-羟基-2-氧代己二酸酯(4-carboxy-4-hydroxy-2-oxoadipate, CHA),进入三羧酸循环(tricarboxylic acid cycle, TCA)。(3) 阿魏酸途径,阿魏酸最初通过生物转化转化为 VAN;而后转化为 PCA,进入 PCA 降解途径。(4) 对香豆酸降解途径,对香豆酸(4-hydroxycinnamic acid, HCNA)转化为 4-羟基苯甲酸钠,随后进入 PCA 降解。(5) 丁香醛降解途径,丁香醛(syringaldehyde, SA)首先转化为丁香酸盐,随后在丁香酸脱甲基酶的作用下转化为 3-O-没食子酸甲酯(3-O-methylgallate, 3MGA),化合物 3MGA 转化为没食子酸盐,观察到的肉桂酸与对羟基苯基单元的氧化有关,这些是芥子基紫丁香基单元的基本构建单元,芥子基单元是木质素结构的一部分。

木质素降解的第一步通常由外源性氧化还原酶如 Lac、LiP、MnP、DyP 完成;随后,产生的异质混合物的芳香族产物通过各种胞内酶进行代谢,包括双加氧酶、脱羧酶、甲基转移酶、水解酶、脱氢酶、超氧化物歧化酶和谷胱甘肽转移酶(与这些酶相关的基因在环状基因组中的表达和定位如图 2B 所示),这些胞内酶将芳香族产物转化为中心中间体;最后,中间体通过 β -酮戊二酸途径进行环裂解并进一步分解进入中心碳代谢;其中,最重要的机制是通过上游途径产生中间产物,如香草醛、苯甲酸和原儿茶酸等,随后,中间产物通过双加氧酶环裂解反应进入下游途径,最终进入 TCA 途径^[63–65,67]。

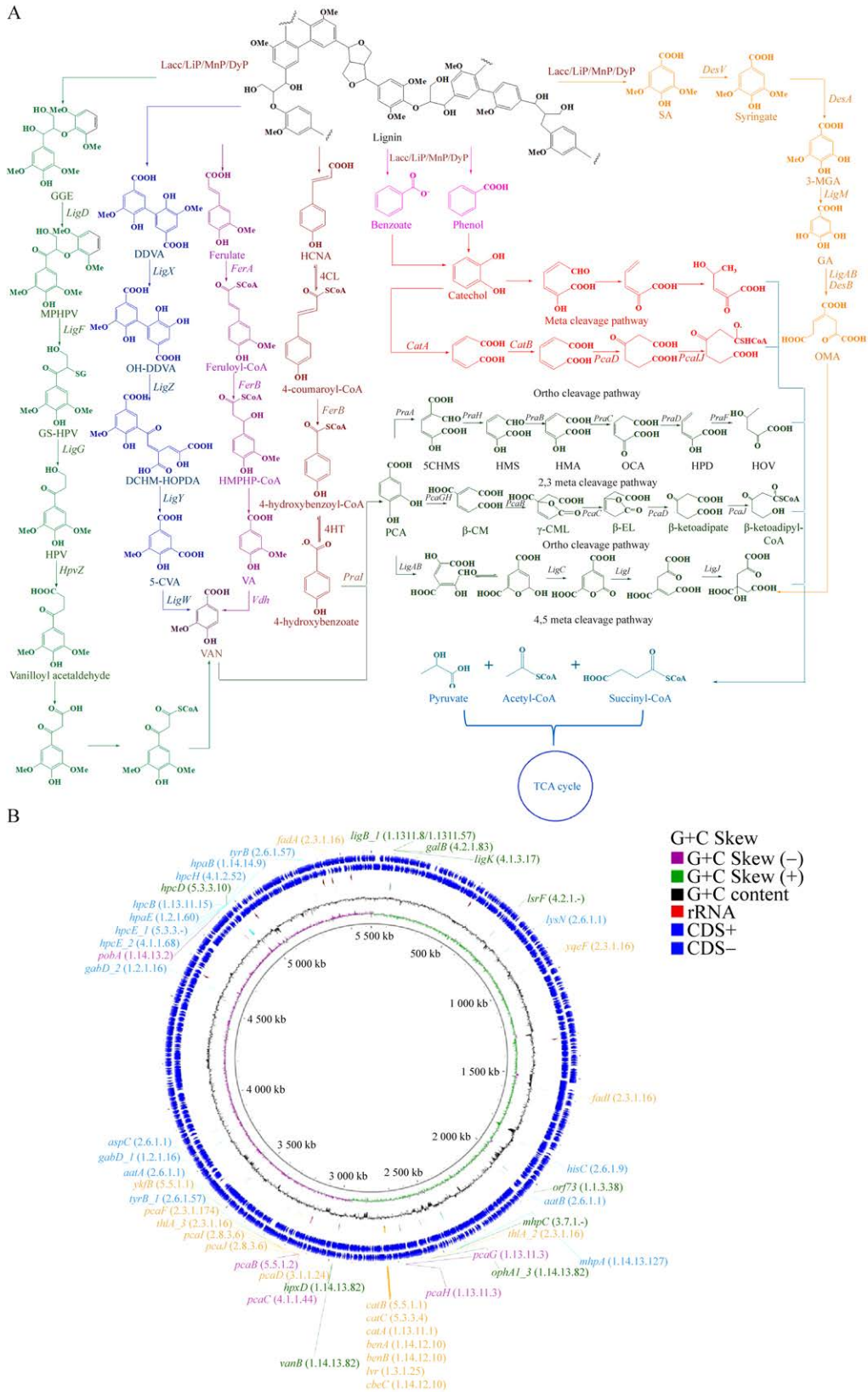


图 2 木质素降解的代谢途径及相关基因的表达和定位(图片改编自参考文献[12]和[67])

Figure 2 Metabolic pathways of lignin degradation and the expression and localisation of their associated genes. A: Metabolic pathway programme for lignin degradation. B: Circular genomic representation and position of the genes present in the lignin fragment degradation model of *Klebsiella variicola* P1CD1. (1) Abbreviation of the substrates and metabolites: CHA, 4-carboxy-4-hydroxy-2-oxoadipate; β -CM, β -carboxymuconate; γ -CML, γ -carboxymuconolactone; 5CVA, 5-carboxyvanillic acid; 5CHMS, 5-carboxy-2-hydroxymuconate-6-semialdehyde; β -EL, β -keto adipate enol-lactone; GA, gallate; GGE, guaiacylglycerol- β -guaiacyl ether; GS-HPV, α -glutathionyl-HPV; DCHM-HOPDA, 4,11-dicarboxy-8-hydroxy-9-methoxy-2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate; VA, vanillin; 3MGA, 3-O-methylgallate; HCNA, 4-hydroxycinnamic acid; HMA, 2-hydroxymuconate; HMS, 2-hydroxymuconate-6-semialdehyde; HOV, 4-hydroxy-2-oxo-valerate; OCA, 4-oxalocrotonate; HPD, 2-hydroxypenta-2,4-dienoate; HPV, β -hydroxypropiovanillone; OMA, 4-oxalomesaconate; MPHPV, α -(2-methoxyphenoxy)- β -hydroxypropiovanillone; PCA, protocatechuate; OH-DDVA, 2,2'-3-trihydroxy-3'-methoxy-5,5'-dicarboxybiphenyl; PDC, 2-pyrone-4,6-dicarboxylate; VAD, vanilloyl acetic acid; VAA-CoA, 4-hydroxy-3-methoxyphenyl-b-ketopropionyl-CoA. (2) Abbreviation of the enzymes: CatA, catechol 1,2-dioxygenase; CatB, cis,cis-muconate lactonizing enzyme; DesA, syringate odemethylase; DesB, gallate dioxygenase; DesV, benzaldehyde-derivatives dehydrogenase; DyP, dye-decolorizing peroxidase; FerA, feruloyl-CoA synthetase; FerB, feruloyl-CoA hydratase/lyase; HMSH, 2-hydroxymuconic semialdehyde hydrolase; HpvZ, glucose-methanol-choline oxidoreductase; Lacc, laccase; LigAB, protocatechuate 4,5-dioxygenase; LigC, CHMS dehydrogenase; LigD, C α -dehydrogenase; LigF, β -etherase; LigG, glutathione removing enzyme; LigI, PDC hydrolase; LigJ, OMA hydratase; LigM, vanillate/3-O-methylgallate O-demethylase; LigX, DDVA-demethylase; LigY, OH-DDVA meta-cleavage compound hydrolase; LigZ, OH-DDVA dioxygenase; LigW, 5-carboxyvanillate decarboxylase; LiP, lignin peroxidase; PcaB, β -carboxy-cis,cis-muconate dehydrogenase; PcaC, 4-carboxymuconolactone decarboxylase; PcaD, muconolactone isomerase; MnP, manganese peroxidase; PcaGH, protocatechuate 3,4-dioxygenase; PcaIJ, succinyl-coenzyme A transferase; pheA, phenol hydroxylase; praA, protocatechuate 2,3-dioxygenase; praB, 2-hydroxymuconic semialdehyde dehydrogenase; praC, 4-oxalocrotonate tautomerase; praD, 4-oxalocrotonate decarboxylase; praE, 2-oxopent-4-enoate hydratase; praF, 4-hydroxy-2-oxovalerate aldolase/hydratase; praH, 5-carboxy-2-hydroxymuconate-6-semialdehyde decarboxylase; praI, 4-hydroxybenzoate-3-monooxygenase; Vdh, vanillin dehydrogenase.

4 多组学技术筛选极端微生物降解木质素

经典的依赖适宜条件培养的方法只能识别某一环境中 10%–20% 的微生物群落。相比之下, 包括宏基因组学、宏转录组学、宏蛋白质组学和代谢组学在内的无需对微生物进行分离培养的现代技术, 使得我们能够对不同环境中的微生物群落的木质素降解机制进行探究^[68]。宏基因组学, 也称为微生物环境基因组学, 需要从环境样本中发现的整个微生物种群中直接提取 DNA。此后, 构建宏基因组文库并使用基因组研究策略来研究这些微生物的遗传组成和

群落功能。宏基因组学于 1998 年首次提出, 最初用于土壤和海洋微生物的分类和鉴定, 后来推广用于其他环境, 有助于发现新的微生物物种和遗传元素^[69–70]。宏转录组学是对微生物 RNA 的综合分析, 以研究特定时间间隔内的全基因组转录活性和调控机制^[71]。与宏基因组学不同, 宏转录组学可以识别代谢活跃的微生物群体, 并揭示微生物生长过程中的通用基因表达模式^[72]。宏蛋白质组学是特定时间点环境中微生物组的总蛋白质补充。虽然宏基因组学可以揭示微生物群落中潜在基因的功能, 宏转录组学可以深入了解基因表达和活性, 但其细胞定位和活性调节发生在蛋白质水平, 因此从转

录组和蛋白质组获得的信息会有所不同^[73], 蛋白质组学是一种用于表征细胞、组织或生物体中存在的蛋白质并生成蛋白质表达定量图谱的方法。蛋白质组学分析可以揭示生物体的实际表达谱, 这是使用纯基因组方法无法实现的^[74]。代谢组学是一种基于核磁共振(nuclear magnetic resonance, NMR)、气相色谱-质谱(GC-MS)、液相色谱-质谱(liquid chromatography-mass spectrometry, LC-MS)、毛细管电泳-质谱(capillary electrophoresis-mass spectrometry, CE-MS)的新兴技术^[75]。代谢组学分析是对生物系统中初级和次级代谢物进行检测、分类和定量的完整过程, 使得复杂基因和蛋白质网络的研究变得简单直观, 代谢组分析是一种强大的工具, 可以帮助

确定微生物降解木质素过程中产生的小分子的种类和丰度, 与基因和蛋白质相比, 代谢物的产生是生物体中最下游的活动^[76]。使用单一方法来调查高度复杂的微生物群落将导致描述不完整。为了克服这个问题, 许多研究人员正在利用整合宏基因组学、宏转录组学、宏蛋白质组学和代谢组学的多组学方法来研究某一环境中的微生物群落(各组学之间的关系如图 3 所示)。结合宏基因组和宏蛋白质组分析, 可以描述在精确条件下共同生长过程中产生的关键酶, 而不仅仅是识别微生物群落的遗传信息。此外, 宏基因组学和宏转录组学能够预测微生物群的功能潜力, 而无需完整的基因组重建。总而言之, 通过整合这些不同层面的数据, 可以更全面地

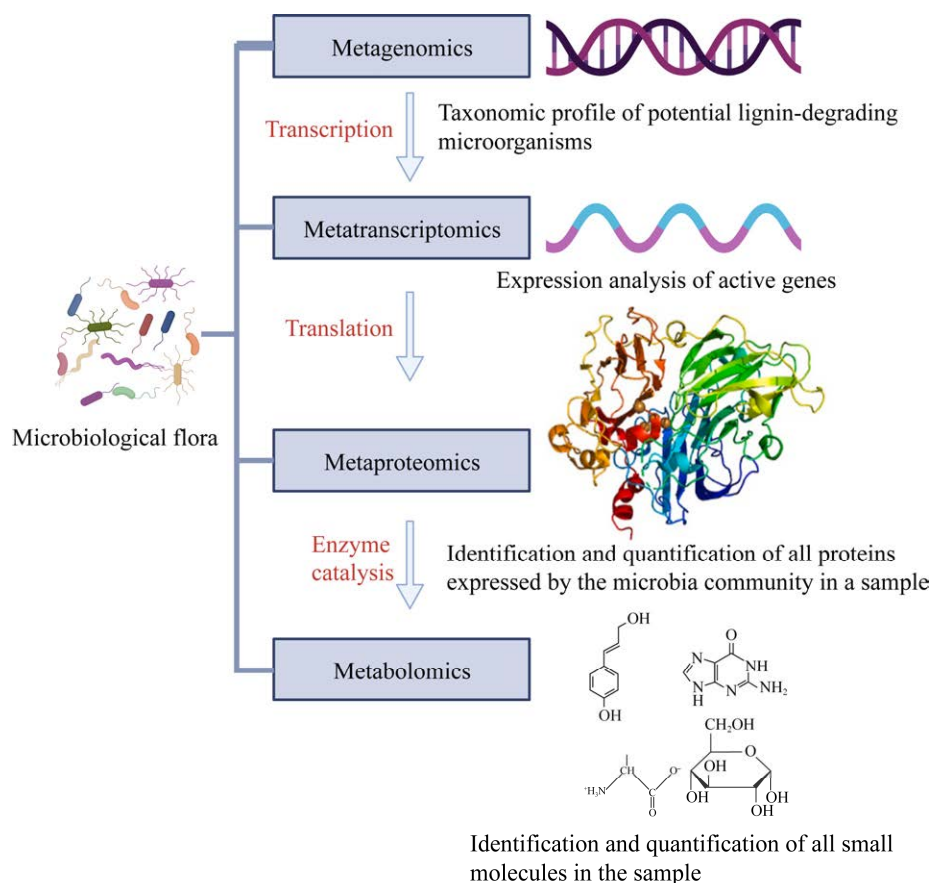


图 3 组学关系图

Figure 3 Omics relationship diagram.

解微生物群落的结构、功能,从而促进微生物的相关应用。

Hassa 等^[77]通过整合宏基因组测序、宏转录组学、宏蛋白组学和代谢组学方法,对沼气发酵中的微生物群落进行分析,深入了解了其微生物群落的组成、性能、群落成员之间的相互关系,这为厌氧发酵条件下木质纤维素降解菌的筛选奠定了基础。Tang 等^[78]的研究发现,竹节虫(*Cyrtotrachelus buqueti*)的肠道分泌蛋白的纤维素降解率为 30.23%–38.24%,半纤维素为 58.56%–70.96%,木质素为 28.71%–43.43%;而微生物群的纤维素降解率为 28.41%–38.31%,半纤维素为 53.45%–66.15%,木质素为 25.65%–38.37%;该研究综合宏基因组测序和转录组测序分析确定了核心微生物群落和碳水化合物活性酶(carbohydrate-active enzyme, CAZymes),显示出微生物组和转录组的密切相关性,揭示了竹节虫肠道微生物降解木质纤维素的机制。此外,还可以利用宏基因组测序技术分析已有的耐低温秸秆降解菌群之间的相关性,对其进行优化,降低菌群的复杂性,以增强实际应用中的优势^[79]。Wang 等^[80]基于全基因组分析的宏基因组测序解释了玉米秸秆和猪粪共消化和单一消化中微生物群落的分类和功能模式,发现耐热梭状芽孢杆菌(*Clostridium stercorarium*)是玉米秸秆降解的特异菌种,甲烷鬃毛状菌(*Methanosaeta concilii*)是主要的产甲烷菌种。D'haeseleer 等^[81]基于蛋白质组学和宏基因组测序技术分析了解构柳枝稷[*Panicum virgatum* (可作为生物燃料)]的嗜热微生物群,检测到了许多种类的木质素降解酶,并证实了类芽孢杆菌(*Paenibacillus*)在生物质解构中发挥着核心作用。Martien 和 Amador-Noguez^[82]指出代谢组学分析可以揭示目标基因,表征木质素等微生物燃料的代谢过

程,从而实现木质素原料的利用,并提高工程微生物的应激耐受性。

由于极端微生物及其酶的特殊生理特征以及缺乏定制的分子生物学工具,研究这些微生物比普通微生物更具挑战性。基于宏基因组学、转录组学、蛋白质组学和代谢组学的微生物鉴定方法,打破了依赖于微生物分离培养方法的严格界限,使得能够对由不可培养和可培养微生物组成的任何环境样本进行深入研究。此类技术允许探测和分析大量数据,这些数据代表任何生物系统在 DNA、RNA、蛋白质和代谢物水平上的整体功能和结构^[83]。综上所述,组学技术为筛选和鉴定降解木质素的极端微生物和极端酶提供了新的方向。

5 小结与展望

木质素资源丰富,但结构复杂,由酚丙烷单元组成,通过不同的键合方式连接,导致其降解效率受限。近年来利用高通量测序技术以及多组学技术已经筛选出了许多木质素降解菌,并对其降解机制有了一定的了解。然而,木质素的工业应用往往需要特定的环境条件,如高温高压、极端 pH 等。极端微生物在恶劣的条件下仍能高效地分泌极端酶来特异性地断裂这些键合,从而降解木质素。因此,对极端微生物的开发和利用是在未来的研究中需要加强的,具体应侧重以下方面。

(1) 丰富极端微生物资源库。应当继续从不同的环境中筛选能够降解木质素的极端微生物,如冻土、热泉、深海以及食草动物的胃肠道等。同时,将筛选得到的微生物进行适应性进化,在实验室模拟极端微生物在自然环境中的进化过程,筛选出具有更强适应性和生产性能的菌株^[84]。

(2) 构建降解木质素的人工复合菌群。单一微生物菌株往往难以完全降解木质素, 通过构建复合菌群, 可以将分泌不同类型木质素降解酶的微生物组合在一起, 这些酶可以协同作用, 打破木质素的不同化学键, 从而提高降解效率。复合菌群中的微生物可能具有不同的环境适应性, 这样的组合可以使复合菌群在更广泛的环境条件下稳定工作, 增强了木质素降解过程的稳健性; 不同的微生物可能对木质素的不同部分有不同的降解偏好。通过合理组合, 可以针对特定的木质素结构设计复合菌群, 提高对目标化合物的选择性降解, 减少不必要的副产物生成。相比于化学法, 生物法降解木质素通常更为环保且成本较低; 通过优化复合菌群的构建, 可以减少对化学助剂的依赖, 降低生产成本, 同时减少化学处理可能带来的环境污染。

(3) 对筛选出的极端微生物和极端酶进行生物技术修饰。基因工程改造是通过基因编辑技术, 如 CRISPR/Cas9 系统, 可以对极端微生物的基因组进行精确的修饰, 增强其在特定环境下的适应性或提高极端酶的催化效率^[85]。蛋白质工程是利用理性设计或定向进化的方法, 可以改变极端酶的氨基酸序列, 从而改善其热稳定性、pH 耐受性或底物特异性^[86]。代谢工程是通过增加碳源前体、敲除旁路代谢途径、减少副产物生成、提高目标产物的转运等策略, 优化极端微生物的代谢途径, 提高其生产效率和产物的产率^[87]。微生物表面显示技术是将极端酶或其编码基因整合到微生物细胞表面蛋白上, 使酶直接展示在细胞表面, 这样可以在不分离酶的情况下进行催化反应, 简化了反应过程并提高了酶的稳定性^[88]。

(4) 应大规模对木质素分解极端微生物进行全基因组测序, 然后进行比较基因组分析和基因组挖掘, 以确定木质素降解和生物漏斗路

径、转录调节因子和限速机制。此外, 注重木质素分解极端微生物的转录组学、蛋白质组学和代谢组学综合分析, 揭示木质素的生物降解功能和机制。

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