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木质纤维生物质资源高效利用分子技术的开发

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摘　要：木质纤维生物质是地球上最丰富的可再生生物质资源，可为造纸、化工、纺织和生物能源等工业提供重要的原材料。木质纤维生物质主要包括木质素、纤维素和半纤维素三种生物多聚物成分。如何利用分子手段改造这些生物聚合物，提高它们的工业利用率是目前高度关注的问题。综述了近年来木质纤维多聚物在生物合成与改造方面的研究进展，展望了利用分子技术改造植物木质纤维生物质实现其高效利用的前景。

关键词：木质纤维生物质；木质素；纤维素；半纤维素

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Molecular technology for high efficient utilization of lignocellulosic biomass

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Abstract: Lignocellulosic biomass, the most abundant renewable resources on the earth, provides main raw materials for production of paper, chemicals, textile products, bioenergy and so on. Lignocellulosic biomass mainly consists of three biopolymers: cellulose, hemicellulose and lignin. The utilization of these biopolymers is determined by their compositional and structural properties. Modification of the biopolymers through molecular technology promises more efficient utilization of lignocellulosic biomass. This review summaries the recent progress on the biosynthesis and molecular modification of three biopolymers and offers perspectives of molecular technology for lignocellulosic biomass modification.

Key words: lignocellulosic biomass; lignin; cellulose; hemicellulose

植物通过光合作用所积累的生物质包括糖类、油脂和木质纤维类生物质。木质纤维生物质，占地球上整个植物生物质的 90% 以上，是最丰富的可再生生物质资源。近年来，人口增长、化石资源匮乏和环境污染之间的矛盾促使人们将目光投向了可再生木质纤维生物质资源的开发和利用。由于目前木质纤维生物质的利用率比较低，开发木质纤维生物质高效利用的新技术变得十分迫切。随着木质纤维生物质合成等科学问题研究的逐步深入，分子改造木质纤维生物质已成为现实，并将从根本上极大提高木质纤维生物质的工业利用率。

1　木质纤维生物质的组成及其结构

木质纤维生物质是植物利用光合作用将太阳能固定在植物细胞壁中的主要生物质。木质纤维生物质主要包含纤维素 (约 39%~45%)、半纤维素 (20%~30%) 和木质素 (22%~31%)[1]。这些多聚物的组成和结构是不同的。

1.1　纤维素

纤维素是地球上含量最高的木质纤维多聚物，在高等植物的细胞壁中以微纤丝 (microfibril) 的形式存在。微纤丝推测由线性的 36 条 β-1,4- 葡聚糖链构成 [2]。每条葡聚糖链由几千到上万个单糖分子

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组成。糖链中葡萄糖单糖分子的数目称为纤维素的聚合度 (degree of polymerization, DP)。葡聚糖链通过链内和链间的氢键形成结晶区。结晶部分占纤维素总量的百分比称为结晶度 (crystallinity)。受遗传和环境的影响，木材中纤维素的结晶度大约为 50%~70% [3-5]。

1.2 半纤维素

与纤维素不同，半纤维素是由不同单糖分子组成的具有分支侧链的杂多糖。半纤维素以无定型状态存在，其聚合度较小，为 50~300 [6]。从进化上看，甘露聚糖是低等植物轮藻细胞壁中最主要的半纤维素。在苔藓、石松类植物和裸子植物中甘露聚糖的含量也比较多。针叶树主要的半纤维素是半乳糖葡糖甘露聚糖 (galactoglucomannan, GGM；约占半纤维总量的 10%~30%)，并含有少量的葡糖醛酸阿拉伯木聚糖 (glucuronoarabinoxylan, GAX；占半纤维总量的 5%~15%)[7-8]。而双子叶植物中主要含有葡糖醛酸木聚糖 (glucuronoxylan, GX；占半纤维总量的 20%~30%)。单子叶植物最主要的半纤维素是葡糖醛酸阿拉伯木聚糖 (GAX)，占半纤维总量的 40%~50% [7]。

结构上，葡糖醛酸木聚糖 (GX) 的主链为 β-1,4-木聚糖链，木糖的 C2 和 C3 位置经常发生乙酰化 (3.5~7 乙酰基 /10 木糖)，侧链为 1,2 连接的 4-O-甲基 -α-D- 葡糖醛酸基团。葡糖醛酸基团可以和木质素单体通过酯键发生共价交联形成木质素 - 碳水化合物复合物 (LCCs)[9]。葡糖醛酸阿拉伯木聚糖 (GAX) 的结构与葡糖醛酸木聚糖 (GX) 类似，不同的是侧链上具有 1,3 连接的 α-L- 阿拉伯呋喃糖基团。其中阿拉伯糖 : 糖醛酸 : 木糖的比例约为 1:2:8。阿拉伯糖经常发生阿魏酸酯化。阿魏酸酯基可以通过氧化进行分子内和分子间交联，也可以和木质素单体进行交联 [10]。另外，四聚寡糖 β-D-Xyl-(1,3)-α-L-Rha-(1,2)-α-D-GalA-(1,4)-D-Xyl 被发现存在于双子叶植物葡糖醛酸木聚糖 (GX) 的还原端 [11-13]，但是在草本植物的阿拉伯木聚糖 (GAX) 中却不存在 [14]。半乳糖葡糖甘露聚糖 (GGM) 的主链是 1,4 连接的 β-D- 葡萄糖和 D- 甘露糖，甘露糖的 C2 和 C3 位置发生部分乙酰化，侧链含有 1,6 连接 α-D- 半乳糖 (图 1)。

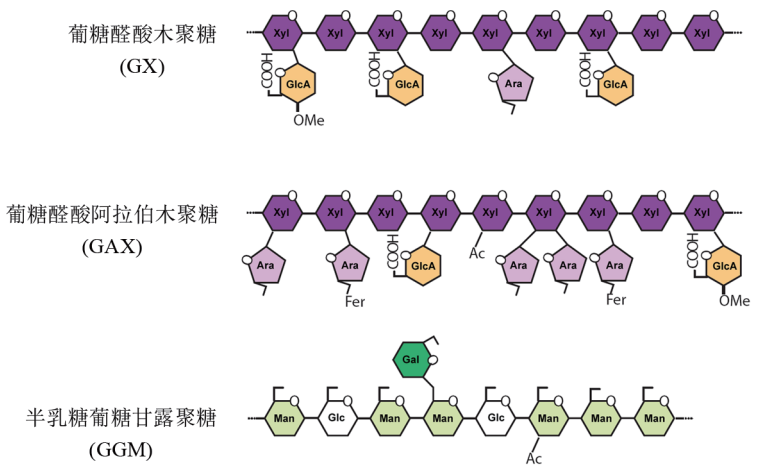
1.3 木质素

木质素在化学组成上区别于上述两种多聚物，它是多酚类物质的聚合物。被子植物木质素主要是由 G 木质素单体松柏醇 (coniferyl alcohol) 和 S 木质素单体芥子醇 (sinapyl alcohol) 聚合而成的，S/G 约为 2.2 [15]。裸子植物木质素单体主要是 G 木质素单体松柏醇，并含有少量 H 木质素单体对 - 香豆醇 (*p*-coumaryl alcohol)。单子叶植物水稻中含有 3 种木质素单体，其比例约为 H:G:S = 1:7:2 [16]。目前认为木质素单体在细胞壁内相互之间通过氧化交联形成多样共价键 [17]。最近也有研究报道称木质素在细胞壁内主要以线性结构存在 [18-19]，但是木质素的具体结构目前仍不清楚。

2 木质纤维生物质的生物合成及分子改造

2.1 纤维素的合成及分子改造

目前认为，纤维素是由位于质膜上玫瑰花型结



Xyl：D-木糖；GlcA：D-葡糖醛酸；Ara：L-阿拉伯糖；Man：D-甘露聚糖；Glc：D-葡萄糖；Gal：D-半乳糖； Ac：乙酰基；Me：甲基；Fer：阿魏酸酯基。

图1 半纤维素分子结构图(修改自[7])

构的纤维素合酶复合体 (CSC) 合成的[20]。纤维素合酶复合体包含 6 个亚基，每个亚基含有 6 个 CesA 蛋白，36 个 CesA 蛋白合成 36 条糖链并通过氢键形成一条微纤丝[21]。CesA 蛋白属于糖苷转移酶家族蛋白 (glycosyltransferase, GT)，它以 UDP- 葡萄糖为底物。由于纤维素合酶复合体结构的复杂性，其生化活性目前尚未鉴定。

对拟南芥 *CesA* 基因的研究发现，两类纤维素合酶复合体分别参与初生细胞壁和次生细胞壁纤维素的合成。由 AtCesA1、AtCesA3 和 AtCesA6/AtCesA2/AtCesA5/AtCesA9 组成的 I 类纤维素合酶复合体主要负责初生细胞壁中纤维素的合成[22-26]。同时，它们也参与表皮毛厚壁细胞和种皮表皮厚壁细胞中纤维素的合成[27-28]。由 AtCesA4、AtCesA7 和 AtCesA8 组成的 II 类纤维素合酶复合体主要负责维管组织次生细胞壁中纤维素的合成[29-32]。拟南芥两类 CesA 蛋白还可以组成混合复合体在特定时期发挥作用[33]。对杨树次生维管细胞壁合成机制的解析发现两类纤维素合酶复合体，它们共同参与了次生细胞壁纤维素的合成。组成这两类复合体的 CesA 蛋白分别与拟南芥两类复合体的 CesA 蛋白同源[34]。初生细胞壁纤维素的结晶度和聚合度比较低，而次生细胞壁中的比较高，这暗示杨树一类纤维素合酶复合体参与低结晶度和聚合度纤维素的合成，另一类参与高结晶度和聚合度纤维素的合成[34]。另外，拟南芥次生细胞壁合成相关 *CesA* 基因的表达受到转录因子 MYB46 的直接调控[35]。SUSY、KOR、COBRA、FRA1、KOBITO、CTL1/POM1/ELP1/HOT2、POM2 和 CSI1 等蛋白也参与纤维素的合成[36-45]。它们为纤维素的合成提供所需底物，并在调节微纤丝在细胞壁上的沉积，引导微纤丝的排布等过程中发挥重要的作用。

纤维素是木质纤维生物中的主要利用成分，但其结晶结构阻碍纤维素酶对其进行水解。提高纤维素的含量，降低其结晶度是纤维素分子改造的方向。KOR 蛋白是 γ 类的 β-1,4- 葡聚糖酶。过表达 *Kor* 基因可以提高纤维素含量并降低纤维素的结晶度[38,46]。SUSY 蛋白是蔗糖合酶，为纤维素的合成提供底物 UDP- 葡萄糖。在杨树中过表达 *Susy* 基因可以提高纤维素的含量，但同时结晶度也有所提高[47]。本课题组的研究暗示了如果通过分子手段上调低结晶度合成相关纤维素合酶复合体的表达，下调高结晶度合成相关纤维素合酶复合体的表达，将有助于在不影响纤维素含量的情况下降低纤维素结晶度，提高纤维素的利用效率，但还需要进行深入研究[34]。

2.2　半纤维素的合成及分子改造

木聚糖是含量仅次于纤维素的半纤维素。木聚糖的骨架与纤维素的骨架都是由 β-1,4 键连接的单一多糖分子。因此推测，木聚糖的骨架可能是由 CesA 家族类似蛋白 (CSL) 合成的。然而，到目前为止，尚未发现 CSL 蛋白参与木聚糖骨架的合成。在对拟南芥突变体的分析中发现，GT43 家族蛋白 (IRX9 和 IRX14) 和 GT47 家族蛋白 (IRX10 和 IRX10-like) 参与了木聚糖骨架的合成，但是利用大肠杆菌和烟草细胞在体外表达这些酶，却没有检测到木聚糖合酶的活性[7,48-52]。在小麦中，GT43、GT47 和 GT75 家族的蛋白组成蛋白质复合体共同参与木聚糖的合成[53]。木聚糖还原端的寡糖分子是由 GT8 家族蛋白 IRX8 和 PARVUS 以及 GT43 家族蛋白 IRX9 参与合成的[11,54-55]。DUF231 家族蛋白参与修饰木聚糖主链木糖的 2-O- 和 3-O- 乙酰化[56]。GT8 家族的 GUX 蛋白参与合成木聚糖的葡糖醛酸侧链[57-59]。DUF579 家族 GXMT 蛋白修饰葡糖醛酸侧链的 4-O- 甲基化[60-61]。草本植物木聚糖 α-(1,3) 阿拉伯糖侧链则由 GT61 家族蛋白参与合成[62]。甘露聚糖骨架的合成是由 CSLA 家族的糖基转移酶完成的[63]。CSLA 能够同时利用 GDP- 甘露糖和 GDP- 葡萄糖作为底物[64-65]。CSLD 家族的蛋白也参与甘露聚糖的合成[7]。甘露聚糖的半乳糖基侧链由 GT34 家族蛋白参与合成[66-69]。

木糖属于五碳糖。在发酵过程中酵母对五碳糖的代谢能力低，而且还受木糖降解产物的抑制。同时，半纤维素的侧链阻碍了水解，乙酰化修饰则影响糖发酵[70]。降低纤维生物质中木聚糖的含量，适当去除侧链和修饰有助于提高半纤维素的利用率。降低木聚糖的侧链含量，可以提高纤维生物质的糖化程度[58-71]。木聚糖合成的底物是 UDP- 木糖，由葡萄糖醛酸脱羧酶提供，在烟草中降低该类基因的表达，可以使植物的木糖总量下降 20%[72]。降低杨树中 *GT47*、*GT8* 和 *GT43* 等参与木聚糖合成的基因可以降低木糖的含量，同时，还可以显著提高木材的降解效率[55,73]。另外，从进化上来看，木聚糖是被子植物中最主要的半纤维素，而甘露聚糖是裸子植物中最主要的半纤维素。它们之间有一定的转换机制[7]。如果能够了解这一机制，将有助于在植物体内降低木聚糖含量，提高甘露聚糖含量，从而解决发酵过程中五碳糖降解效率的问题。

2.3 木质素的合成及分子改造

木质素单体在细胞质中由苯丙氨酸通过一系列的酶促反应形成 (图 2)，这些酶包括 PAL、C4H、C3H、4CL、CoAOMT、CCR、Cald5H/F5H、AldOMT、CAD 和 SAD [74-75]。其中 4CL 被认为是控制木质素总量的关键因子，Cald5H 是控制木质素 S/G 比例的关键因子。这些酶的基因受转录因子 MYB58、MYB63 和 MYB85 的直接调控 [76-77]。合成的木质素单体经历糖基化修饰 [78-79]，并通过 ABC 转运蛋白运输到细胞壁 [80]，最终木质素单体在细胞壁中进行氧化聚合形成木质素 [81-83]。目前木质素单体的合成已经比较清楚，但是木质素单体的糖基化修饰、运输和聚合等还有待深入研究。

木质素在细胞壁中与半纤维素相互交联，影响了纤维素和半纤维素的释放。降低木质素的含量，提高木质素的松散度可以提高木质纤维生物质的利用率。目前木质素生物合成代谢途径已得到充分解析，在此基础上建立的生物技术在改造木质素含量与木质素单体组成方面已被多个实验室证实。木质素单体之间经常通过 β-O-4、α-O-4、4-O-5 或 β-5/α-O-4 等键进行连接 [17]。在拟南芥中过表达木质素单体 4-O- 甲基转移酶阻止这些键的形成，可以降低木质素单体的聚合度，茎秆糖化效率提高约 22%[84]。在杨树中，利用转基因技术，反义降低 *4CL* 基因的表达，发现木质素含量能降低到 50%，同时，纤维素含量增加。利用多基因转化系统，在降低 *4CL* 基因表达的同时提高 *AldOMT* 基因的表达，可以使木质素含量降低达 50%，单体组成的 S/G 比率从 2 增加到 6，木质素松散度增加，纤维素含量增加 30%[15,85-86]。通过抑制 *CCR* 基因的表达，木质素含量也可以降低达 50%。对田间试验种植 5 年的转基因杨树分析表明，转基因植物材料的木质素降解效率提高；同时也观察到木质素含量如降低太多，植物生长可能会受到影响 [87]。

3 展望

近几年来，利用木质纤维生物质转化成方便使用的液体燃料受到了极大的关注，被认为是第二代生物质能源的发展方向。多种植物，如芒草、柳枝稷、杨树和桉树等，被广泛看作是最具潜力的生物质能源植物 [88-90]。纤维素燃料乙醇生产过程主要包括预处理、酶解和发酵三大关键步骤。目前木质纤维生物质预处理过程需要进行高温高压爆破、酸碱处理等物理化学过程，目的是打破纤维素、半纤维素和木质素之间的连接，去除木质素，降低纤维素结晶度使其适于酶解。这些预处理过程具有操作危险、能耗高、形成发酵抑制物、造成环境污染、需耐腐蚀反应装置等缺点。如何解决目前预处理的高能耗和低效率，提高发酵过程的五碳糖利用率等，是该工艺商业化的主要制约因素。采用分子手段改造纤维生物质的组成和结构，使木质纤维素在温和条件下进行酶解并提高产出，是木质纤维生物质资源高

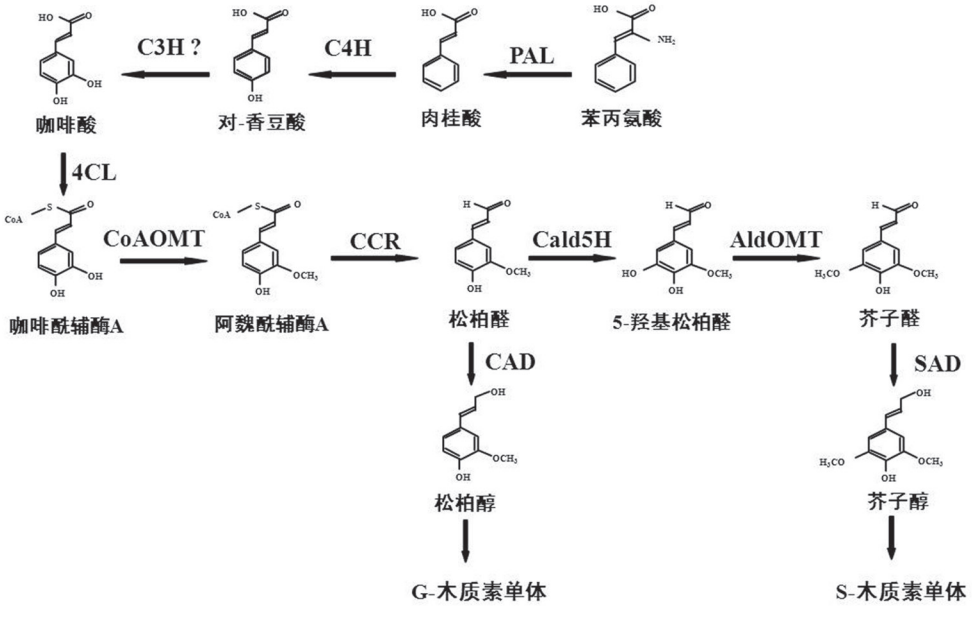


图2 杨树木质素单体合成途径

效利用新的探索方向。

然而，开发提高生物质转化利用率的分子新技术仍然缺乏一些理论基础。如需要搞清纤维素酶复合体怎样组装和工作，合成过程中结晶结构怎样调节；木聚糖合成相关的复杂酶系如何工作；在降低植物木质素总量的情况下，如何才能不影响植物导管水分运输；三大木质纤维组分协调合成的网络调控机制等。这些问题的解析将有助于定向设计可高效利用的生物质新品系，并推动生物质能源的开发。

目前多个实验室在探索利用生物技术对柳枝稷和玉米秆等进行基因工程改造，并提高了生物质的转化效率[91-92]。如降低柳枝稷 *COMT* 基因表达，可以适度下调木质素的含量，同时改变 G 型和 S 型木质素的含量。利用传统的发酵方法就可以使改良后柳枝稷的乙醇产量提高 38%[93]。另外，在探索培育可自降解的生物质原料方面，对玉米和烟草的研究目前取得了一定的进展[94-95]。在玉米液泡中表达纤维放线菌 (*Acidothermus cellulolyticus*) 纤维素酶 E1 可以提高生物质的转化，并且对植物没有毒害[96-97]。这项研究为定向培育高效生物质开辟了另一条令人鼓舞的途径。目前生产纤维乙醇的一些基本技术已在多个实验室建立，一些示范工厂在进行中试生产，如加拿大 Iogen 公司 (http://www.iogen.ca) 建立的示范厂年产乙醇 70 万加仑，已运行多年。

采用基因工程手段改造生物质性状并提高生物质转化利用效率是一项新的技术。这类技术包括多聚纤维生物质组分木质素、纤维素和半纤维素合成的基因工程，生物质合成与积累调控的基因工程，生物质结构与能源转化性状的基因改造工程等。目前许多技术的研发还处于实验室和温室阶段，生产应用的许多相关研究亟需进行。开发可高效利用木质纤维生物质的技术对缓解人类发展所面临的能源及环境挑战具有重要意义。

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