# 内切木聚糖酶的应用及其酶制剂开发技术*

冼　亮,秦　艳,朱　婧,李　亿,梁　戈,左晓琼,黄日波,王青艳**
(广西科学院,国家非粮生物质能源工程技术研究中心,非粮生物质酶解国家重点实验室,广西南宁　530007)

**摘要**:木聚糖是自然界中储量第二大的自然多糖。木聚糖酶是水解木聚糖的一系列酶的统称,其中内切木聚糖酶在木聚糖的水解中起至关重要的作用。内切木聚糖酶的用途十分广泛,并且研究者以不同用途为导向对该酶进行了大量研究。本文介绍了内切木聚糖酶的各种用途,以及酶的发现、生产和改性概况,为内切木聚糖酶的应用相关研究提供参考。

**关键词**:内切木聚糖酶　酶制剂　培养方式　农林业废弃物　蛋白质工程　异源表达　转录调控

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## 0　引言

木聚糖是自然界中储量第二大的自然多糖,将其资源化可带来极大利益[1]。木聚糖有一条由木糖以β-1,4-木糖苷键连接而成的主链,部分木糖残基带有短侧链。侧链基团含有乙酰基、阿拉伯糖基、半乳糖基、葡萄糖醛酸基、阿魏酸等的一种或几种,另外,香豆酸也有可能属于木聚糖的侧链基团[2]。

广义的木聚糖酶是水解木聚糖主链和侧链的酶的统称,狭义的木聚糖酶仅指水解木聚糖主链的酶,即内切木聚糖酶(E.C.3.2.1.8)和木糖苷酶(E.C.3.2.1.37)[3]。内切木聚糖酶以随机方式水解主链内部的β-1,4-木糖苷键,是对木聚糖的快速解聚贡献最大的酶,因而吸引了研究者的极大兴趣,并且该酶已经被广泛地应用在多个领域中[4]。

近年来,有关内切木聚糖酶的研究热度不减,在新酶的发现和已有酶的改良、单一酶制剂的发现和应用、复合酶制剂的开发、传统方法改良菌株的产酶量和分子生物学水平的转录调控等方面,均有持续不断的报道。此外,有新结构的木聚糖的发现也带来了相应新的内切木聚糖酶,拓宽了人们对这个古老的酶的认识。现有国内综述多为陈述较早前的研究结果,对近年来国内和国际同行取得的较新研究结果较少涉及,基于此,本文结合近年来的报道,对内切木聚糖酶与应用相关的研究进行简单介绍。

## 1　内切木聚糖酶的分子特征

与大多数酶一样,一个内切木聚糖酶由一个基因编码,由一个 mRNA 翻译而来。内切木聚糖酶一般

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【作者简介】
冼　亮(1983—),男,博士,主要从事生物质水解和食品用酶的开发研究。
【**通信作者】
王青艳(1972—),女,博士,研究员,主要从事应用微生物菌种选育与酶工程研究,E-mail:qingyanw@126.com。
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以单体的形式存在,其分子量低者只有20 kDa[5],高者可超过100 kDa[6]。内切木聚糖酶群体的氨基酸序列的多样性很大,分属于糖苷水解酶第5,6[7],7,8[8],10,11,30[9]和43家族,其中以属于家族10和11的个体为多见[10]。该酶的水解机制为酸碱催化[10]。

## 2 内切木聚糖酶的酶学性质

在内切木聚糖酶群体中,不同个体之间的每一项酶学性质都呈现出很强的多样性,这就使该酶的不同个体可以被用于不同的反应条件中,进而拓宽了该酶的应用范围。已报道内切木聚糖酶的最适作用pH值为4.0[11]—11.0[12],可在pH值为4.0—11.5[13]区段稳定;最适作用温度为25[14]—100℃[15],热稳定性极差[16]和极佳[15]的内切木聚糖酶均有报道。有的内切木聚糖酶只能水解木聚糖[17],而有的却能水解多种底物[18]。内切木聚糖酶对木聚糖的比活力(U/mg蛋白质,国际标准酶活力单位)变化区间极大,有仅为个位数的[6],也有将近百万的[19]。大多数内切木聚糖酶的$K_m$值为1—20 g/L木聚糖。

## 3 内切木聚糖酶的用途

### 3.1 饲料添加剂

内切木聚糖酶的用途很广泛,其中最成熟的用途是作为动物的饲料添加剂,目前已经出台了饲料用木聚糖酶的国家标准[20]。内切木聚糖酶可以将饲料中的木聚糖预水解,提高饲料的适口性,减轻动物的消化压力,提高饲料的消化度。已有多项饲料用木聚糖酶的研究发表,涉及的动物有奶牛[21]、断奶仔猪[22]、幼鸡[23]、鱼类[24]和刺参[25]等。由于植物细胞壁的结构复杂,市售木聚糖酶试剂除了含有内切木聚糖酶之外,还含有纤维素酶和果胶酶等,不同酶之间相互搭配可以达到更好的使用效果[26]。

### 3.2 食品

内切木聚糖酶在食品方面的应用也比较成熟。在烘焙食品方面,内切木聚糖酶可以增加糕点体积、降低硬度和粘度、提升口感和帮助消化[27]。特别是在含有纤维素质原料的食品中,加入内切木聚糖酶后会有更明显的效果提升[28]。在白酒酿酒方面,添加内切木聚糖酶可以促进原料的水解,提高原料的利用率和产品的酒度[29]。内切木聚糖酶也可以促进啤酒糟的水解,降低其粘度,提高滤过率[30]。果汁的生产主要用到果胶酶[31],加入内切木聚糖酶可以起一定的辅助作用[32]。

### 3.3 营养品和甜味剂

内切木聚糖酶在营养品方面也已有实际应用。内切木聚糖酶将木聚糖完全水解后释放出木糖和低聚木糖(主要由木糖构成的寡糖,聚合度为2—7),其中低聚木糖可以促进肠道益生菌的生长,增进肠道健康,改善人体脂质代谢,进而促进人体健康[33]。已有不少学者对低聚木糖的生产进行研究,他们立足于所在区域,选材因地制宜。例如冯家勋教授等以广西产量丰富的甘蔗渣为原料[34],江正强教授等以北方产量丰富的玉米芯为原料生产低聚木糖[35]。除了可用于生产低聚木糖之外,内切木聚糖酶亦可联用木糖苷酶将木聚糖彻底水解为木糖,用于生产木糖醇[36]。低聚木糖[37,38]和木糖醇[36,39]都是有益人体健康的甜味剂,且均已有相关商品出售。

### 3.4 生物质转化

内切木聚糖酶在生物质转化方面的应用一直是热门研究方向。由于不可再生燃料的储量日益减少,所以迫切需要开发可再生燃料[40]。将木聚糖彻底水解为木糖后,可使用微生物将木糖转化为乙醇[41,42]、丙酮[43]、丁醇[44]和油脂[45]等可再生燃料,也可转化为丁二酸等其他化工原料[46]。

木聚糖和其他多糖与纤维素构成的网络结构是植物细胞壁的主要结构之一,木聚糖酶的水解可以增大纤维素与纤维素酶的接触面以提高纤维素酶的水解率[47],纤维素水解产生的葡萄糖可以用于生产第二代燃料乙醇[48]等大宗产品。迄今已有不少内切木聚糖酶对纤维素的水解起促进作用的报道[49,50]。在纤维素质原料的实验室水解中,用各种预处理方法去除部分木质素和部分半纤维素等物质,再联用内切木聚糖酶和纤维素酶就可以实现纤维素质原料的高效水解[34,51]。

### 3.5 造纸

内切木聚糖酶在造纸方面的应用已有不少研究。内切木聚糖酶可以水解纸浆中的木聚糖,改善纸浆的物理性能,使卡帕值下降[52];可以脱除废纸浆上吸附的油墨,使白度提高[53]。使用该酶处理纸浆后再联用化学法处理能进一步提升纸浆的质量,该酶的处理还可以降低漂白用化学用品的用量,减轻环境压力[54]。

## 4 新酶的开发

### 4.1 从可培养和未培养微生物中获得新酶

内切木聚糖酶主要由微生物产生,从环境样品中筛选新的产酶菌株是获得新酶的有效方法[55]。将酶纯化后可评估该酶的应用效果,并可将性能优异的酶的编码基因克隆表达,大量获得该酶[56,57]。如 Zheng 等[58]从棉花秸秆堆中筛选得到一株类芽孢杆菌,从其液态培养液中纯化得到一个高度耐热的内切木聚糖酶。Yang 等[59]从土壤样品中筛选得到一株烟曲霉,其液态培养液水解甘蔗渣碱提取物主要释放出木二糖,进一步从培养液中纯化得到起作用的内切木聚糖酶,并克隆表达其编码基因。He 等[60]从以竹子为原料的造纸厂样品中筛选得到一株链霉菌,并从菌株的培养液中纯化得到一个胞外内切木聚糖酶,其可在 pH 值 3.0—11.0 区间稳定,并克隆表达其编码基因。

除了从可培养微生物中获得新酶外,也可从各种环境所含未培养微生物中直接克隆表达获得新酶[61]。如 Mo 等[62]通过活性筛选的方法,从堆肥样品的宏基因组文库中获得一种新的耐热内切木聚糖酶。

### 4.2 通过蛋白质工程改变野生酶的性质

蛋白质工程可以改变内切木聚糖酶的酶学性质,获得更具应用潜力的酶。例如 Ventorim 等[63]通过蛋白质工程,将野生酶的热稳定性($T_{1/2}$ 50℃为 0.77 h)提升了数倍($T_{1/2}$ 50℃为 29.46 h);Gibbs 等[64]将最适作用 pH 值为 6.6 的野生酶突变,得到最适作用 pH 值为 8.5 的突变体。另一方面,从学术的角度来看,蛋白质工程可以帮助人们认识氨基酸序列和蛋白质结构等对酶活力的影响,拓宽认知,有助于人们对有应用潜力的酶进行改造。

## 5 酶制剂的开发

### 5.1 单一型酶制剂

单一型酶制剂是指主要含某个酶的酶制剂,在造纸方面,高纤维素酶活力会损害纸浆,故适合用主要含内切木聚糖酶的单一型酶制剂[65]。关于富含单一酶的酶制剂的开发,已有的研究多是将某个酶的编码基因在某个缺乏植物细胞壁成分水解酶的宿主中实现异源表达,获得富含该酶的制剂[17]。常见的宿主有大肠杆菌和毕赤酵母等。但此法的产酶成本高,培养工艺较复杂,并且酶的生产往往需要诱导,难以低成本持续高产。通过菌株筛选或选育获得高产菌株是用于生产单一型内切木聚糖酶制剂的好方法,因为这样获得的菌株容易培养,诱导生产目标酶所用成本较低[66,67]。

### 5.2 复合型酶制剂

单一型酶制剂的缺陷非常明显,由于菌株本身的其他细胞大分子聚合物水解酶的活力低,当以多成分水解为目标时(应用于食品、饲料、生物炼制和营养品方面),这些酶制剂的用途会受到限制[68]。复合型酶制剂是指含有多种细胞大分子聚合物水解酶的酶制剂。复合型酶制剂的开发可从菌株入手,筛选新的产酶菌株,获得高产量和兼具不同酶的酶制剂[69];使用传统诱变方法可以使菌株的基因组引入随机突变,从而提高菌株的产酶能力和调节菌株产生其他酶,进而改良原有酶制剂[70-72]。

对产酶调控机理进行研究不但能增进人们对微生物基因表达调控的了解,还能获得优良的产酶菌株,从而得到优良的酶制剂。冯家勋教授、曲音波教授和周志华研究员等团队各自对通过传统诱变获得高产酶的草酸青霉菌株的基因组进行测序,分析在不同产酶能力对应的培养条件下的基因表达差异,寻找目标酶的转录调控因子,通过基因缺失和回补等方法验证,从分子水平上揭示了产酶调控机理,获得了同时含木聚糖酶、纤维素酶和淀粉酶等酶的复合酶制剂[73,74]。突变的草酸青霉菌株可以以多种农林业废弃物为碳源快速生长并高产酶,所产的复合酶可以将纸浆[51]和碱处理甘蔗渣[34]高效糖化,耦合酵母后还可以将糖化液中的单糖转化为乙醇,故这些酶制剂在食品、饲料、生物炼制和营养品方面均具有很强的应用潜力。赵心清教授团队对瑞氏木霉产生物质水解酶的转录调控机制进行研究,也获得了优良的产复合酶的重组菌株[75,76]。

### 5.3 菌株培养产酶制剂的优化

菌株培养基成分和培养条件的优化是提高产量和调节其他酶产量的有效方法。个别因素可能对产酶的影响较大,例如 Zhang 等[77]改变了液态培养基 pH 值和控制溶解氧的量之后,*Streptomyces griseorubens* JSD-1 的木聚糖酶活力提高到优化前的 3.2 倍。Yin 等[78]优化了多个因素后,将 *Kluyveromyces lactis* 的木聚糖酶活力从 16 U/mL 提升至 49 U/mL。除了使用摇瓶培养外,也有报道对使用发酵罐培养的参数进行优化[79,80]。

使用农业废弃物和下脚料为原料可以变废为宝

并节约酶的生产成本[81]。在液态培养中,麦麸是最常用的[79,82-84],玉米芯[82,85]和黄豆粉[85]等也有报道使用,这些原料的配比使用会使培养基的营养更全面,能够获得更好的产酶效果[82,85]。农业废弃物和下脚料也可以用作固态发酵(Solid-state Fermentation)产酶的基质。与液态发酵的方法相比,固态发酵的传质、传热、pH值等参数难以实时控制,但也有可能获得更好的产酶效果[86,87]。

## 6 β-1,3-内切木聚糖酶

研究者从某些海洋藻类的细胞壁中发现一种由木糖以β-1,3-木糖苷键连接而成的多糖,虽然与常见的以β-1,4-连接的木聚糖不同,但该多糖也属于木聚糖[88,89]。在已报道的水解β-1,3-木聚糖的酶中,有底物特异性实验结果表明该酶不能水解β-1,4-木聚糖,亦不能水解Avicel、羧甲基纤维素钠、昆布多糖和甘露聚糖,证实该酶所具有水解β-1,3-木聚糖的功能不是某些酶的兼有功能[90],因此该酶被称为β-1,3-内切木聚糖酶,并拥有专属的酶学编号EC 3.2.1.32。根据氨基酸序列同源性,该酶属于糖苷水解酶家族26[91]。

虽然关于该酶的报道较少,但该酶的应用前景不可忽视[92]。海洋藻类有可观的生物量,能用作生物炼制的材料生产众多有用的化工品[93]。将β-1,3-木聚糖部分水解可产低聚木糖[94],完全水解生成的木糖可以进一步生物转化为其他产品[43-46]。在已有报道中,该酶主要由微生物产生[94,95]。考虑到微生物基因组的多样性,该酶可能广泛存在于多种微生物的基因组中,只是由于人们对该酶的认识不足而未被广泛发现。该酶的不同个体的分子量和各项酶学性质都存在很大差异,随着人们对海洋藻类生物炼制研究的持续进行,对该酶的研究必定取得更多进展[90,94]。

## 7 展望

人们对内切木聚糖酶的研究由来已久,并已取得了很好的成就,但仍旧存在一些亟待解决的问题。

在营养品的生产方面,原材料的处理成本往往占生产成本的较大比例,已有内切木聚糖酶难以直接从原材料高效地生产低聚木糖,而是只能以预处理后的材料[96]或提取物[17,35,97,98]为原料生产。同样的问题也限制了内切木聚糖酶在食品和生物炼制方面的应用[99]。通过蛋白质工程获得高效水解植物细胞壁其他大分子聚合物的内切木聚糖酶应该可以使成本降低,这或许是未来的研究方向。

在蛋白质工程方面存在两个主要问题。一是非催化氨基酸的突变和酶学性质之间的关系仍未十分清楚,目前仍然难以准确评估非催化氨基酸在野生酶中所起的功能和精准预测突变后的结果。在多个报道中,往往仅突变1个氨基酸就可能造成多项酶学性质的改变,并且有可能产生不符合研究人员初衷的突变体[100],这都印证了已有的研究仍然不足。二是知识产权的壁垒,如果使用某些酶学性质优良的酶作为出发酶,通过蛋白质工程进一步改良该项酶学性质会更有实用意义[101],但这往往很难实现。因为现有专利往往保护了某个酶的本身及其少数几个氨基酸的突变体,在保护知识产权的同时也可能会降低非专利权研究人员使用该酶进行蛋白质工程研究的热情。

关于β-1,3-内切木聚糖酶的报道仍然很少,人们对该酶的了解有限,亟待获得新的酶进行研究。在不断获得新酶后,还应对某些可能符合应用要求的酶进行应用试验并评估其作用效果,才能真正实现海洋藻类生物质资源的综合利用。

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# Application of Endoxylanase and Technologies Used for the Development of Enzyme Reagents

XIAN Liang,QIN Yan,ZHU Jing,LI Yi,LIANG Ge,ZUO Xiaoqiong,HUANG Ribo,WANG Qingyan

(National Engineering Research Center for Non-Food Biorefinery,State Key Laboratory of Non-Food Biomass and Enzyme Technology,Guangxi Academy of Sciences,Nanning,Guangxi,530007,China)

**Abstract**:Xylan is the secondary abundant natural polysaccharide in nature. Xylanase is a collective name for a series of enzymes that hydrolyze xylan,in which endoxylanase plays a vital role in the hydrolysis of xylan. Endoxylanase has been widely used in many fields,and abundant researches have been completed for better application of it in these field. The application,discovery,production and modification of endoxylanase are introduced. This paper might provide reference for the researches in application of endoxylanase. This article introduces the various uses of endoxylanase,as well as an overview of the discovery,production and modification of it,and provides a reference for related research on the application of endoxylanase.

**Key words**:endoxylanase,enzyme reagents,cultivation method,agricultural and forestry waste,protein engineering,heterologous expression,expression regulation

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