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青霉纤维素酶基因的表达调控与重组表达研究进展*

Advances in Regulation of Cellulase Gene Expression in Filamentous Fungi *Penicillium* and Recombinant Expression of *Penicillium* Cellulase Genes

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摘要:青霉属菌株可以分泌完整的纤维素酶系,尤其高产β-葡萄糖苷酶(β-glucosidase,BGL,EC 3.2.1.21),弥补了工业菌株里氏木霉(*Trichoderma reesei*)胞外β-葡萄糖苷酶活性低的不足,加上青霉菌株生长速度快等优点,使其产纤维素酶受到越来越多的关注。为了解决以木质纤维素为原料工业生产燃料乙醇所需纤维素酶量大、成本高等难题,深入研究青霉属纤维素酶基因的表达调控和重组表达非常重要。本文就青霉属纤维素酶基因资源、纤维素酶合成调控网络以及纤维素酶基因的重组表达等进行综述与展望。

关键词:青霉　纤维素酶基因资源　纤维素酶合成调控网络　纤维酶基因重组表达

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Abstract: Besides fast growth of *Penicillium* strains as compared to *Trichoderma reesei*, *Penicillium* strains can secret intact cellulase system, especially producing highly β-glucosidase, which can compensate the low activity of extracellular β-glucosidase of *T. reesei*. Therefore, the cellulases from *Penicillium* have attracted the most attention in recent years. To overcome the bottleneck of vast demand and high cost of the cellulases in industrially producing fuel ethanol from lignocelluloses, it is very important to deeply study the regulation of cellulase gene expression in *Penicillium* and recombinant expression of *Penicillium* cellulase genes. This paper reviews the aspects of cellulase gene resources from *Penicillium*, regulatory network of cellulase synthesis in *Penicillium* and high recombinant expression of *Penicillium* cellulase genes.

Key words: *Penicillium*, cellulase gene resource, regulatory network of cellulase synthesis, recombinant expression of cellulase genes

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0　引言

现代工业的迅速发展,导致能源消耗不断增加,预示着将来能源需求也随之增大。但是,当今石油和天然气等不可再生化石能源日趋枯竭,还有环境污染和气候变化等问题日愈严重。因此,大规模开发与利用绿色清洁、可再生生物质能源——生物燃料显得日

益重要。其中,木质纤维素燃料乙醇是最具有发展前景的生物质能源之一。用微生物将木质纤维素转化为葡萄糖再发酵生产燃料乙醇,对解决以粮食或糖类物质为原料生产燃料乙醇引起的与人争粮、与粮争地等主要问题具有重大意义[1]。

广西是中国最大的甘蔗、木薯等种植基地,年产量均占全国60%以上,每年产生至少1360万t甘蔗渣和数量可观的木薯废弃物(秸秆、叶子)。目前,广西甘蔗渣和木薯废弃物等木质纤维素主要用作锅炉燃料、制浆造纸和生产绿色包装材料等,存在资源浪费、环境污染等主要问题[2,3]。《广西非粮生物质能源产业优先发展规划大纲(2011-2015年)》明确了非粮生物质能源产业是广西农业新兴优势产业,确定了广西是全国首个推广使用非粮原料乙醇汽油的省区。由于广西拥有廉价的、丰富的木质纤维素资源,用木质纤维素生产燃料乙醇对广西非粮生物质能源产业的发展具有重要意义。迄今,国际上还没有一家工业规模用木质纤维素为原料生产燃料乙醇的企业,最主要的障碍是纤维素酶水解成本太高,占生产工艺总耗值的20%[4]。

微生物是纤维素酶的最主要来源,纤维素酶是一组复合酶的总称,主要包括:内切葡聚糖酶(endoglucanase,EG,EC 3.2.1.4)、外切葡聚糖酶(exoglucanase,又叫纤维二糖水解酶,cellobiohydrolase,CBH,EC 3.2.1.91)和β-葡萄糖苷酶(β-glucosidase,BGL,EC 3.2.1.21)[5]。用于生产纤维素酶的微生物菌株大多是丝状真菌,例如木霉属(*Trichoderma*)、曲霉属(*Aspergillus*)和青霉属(*Penicillium*)[6,7]。目前,国际上主要用里氏木霉(*Trichoderma reesei*)突变菌株进行工业生产纤维素酶制剂。尽管木霉属菌株的纤维素酶活性较高,但其分泌的纤维素酶系不够完整,胞外BGL活性低,通常需要添加额外的BGL才能高效地降解木质纤维素,增加了成本。

研究发现,青霉属能分泌完整的纤维素酶系,可以解决以上矛盾,是目前最有潜力和里氏木霉相媲美的真菌[7]。但是,传统天然青霉菌株纤维素酶产量以及酶活性还达不到工业生产的要求。因此,详细深入研究青霉属纤维素酶系组成、表达调控等具有重要的理论与应用价值。本文将对青霉属纤维素酶基因资源及其表达调控、以及青霉纤维素酶基因的重组表达的相关研究进展进行综述和分析。

1 青霉纤维素酶基因资源

自然界中,降解木质纤维素的真菌主要集中在丝状真菌木霉属、青霉属和曲霉属。在中国亚热带和热带森林,青霉属和木霉属菌株是优势种群[8]。已报道的分泌纤维素酶的青霉属菌株至少有30种,其中,对草酸青霉(*P. oxalicum*,*POX*)和微紫青霉(*P. janthinellum*,*PJA*)研究最为详细[9,10]。研究表明,青霉属真菌可以作为里氏木霉的替代菌株,应用在第二代木质纤维素燃料乙醇工业生产上。其优点在于(1)青霉属纤维素酶系含有高BGL酶活,例如:具有相等滤纸酶活力的青霉纤维素酶系,降解木质纤维素产生的葡萄糖是里氏木霉的1.5~3.0倍;(2)青霉属纤维素酶系中糖苷水解酶(glycosyl hydrolase,GH)家族7的CBH1比活高,例如:在相同蛋白量的条件下,细疣青霉(*P. verruculosum*)的CBH1与嗜酸栖热菌(*Acidothermus cellulolyticus*)EG协同作用对玉米秸秆和微晶纤维素进行水解,其转化率是里氏木霉CBH1与之协同作用的1.3~1.5倍[7]。

1.1 青霉纤维素酶基因的克隆

已知的青霉纤维素酶基因已超过100个,主要集中在巴西青霉(*P. brasilianum*,*PBR*)、变灰青霉(*P. canescens*,*PCA*)、产黄青霉(*P. chrysogenum*,*PCH*)、皮落青霉(*P. crustosum*,*PCR*)、斜卧青霉(*P. decumbens*,*PDE*)、指状青霉(*P. digitatum*,*PDI*)、棘刺青霉(*P. echinulatum*,*PEC*)、扩展青霉(*P. expansum*,*PEX*)、光滑青霉(*P. glabrum*,*PGL*)、粒状青霉(*P. granulatum*,*PGRA*)、意大利青霉(*P. italicum*,*PIT*)、微紫青霉(*PJA*)、朱黄青霉(*P. minioluteum*,*PMI*)、*P. occitanis*(*POC*)、草酸青霉(*POX*)、产红青霉(*P. rubens*,*PRU*)、娄地青霉(*P. roqueforti*,*PRO*)等(表1~表3)。除少数青霉纤维素酶基因如微紫青霉的*cbh*1[11]外,都含有内含子。与其它真菌纤维素酶基因不同的是,这些内含子的数目、长度和相对位置均无保守性。

表1 已知氨基酸序列的青霉外切葡聚糖酶

Table 1 Exoglucanases with known amino acid sequences from *Penicillium* strains

CAZy家族 CAZy family	酶名称 Enzyme name	来源菌株 Source strain	GenBank Accession number	重组表达 Recombinant expression	参考文献 References
GH6	POXJ CBH2	*P. oxalicum* JU-A10	ADX86895	*P. oxalicum*	[12~14]
	PRU CBH2	*P. rubens* Wisconsin 54-1255	CAP93233.1	—	[15,16]
	PEXM CBH2	*P. expansum* MD-8	KGO54790.1	—	[17]

续表 1
Continue table 1

CAZy 家族 CAZy family	酶名称 Enzyme name	来源菌株 Source strain	GenBank Accession number	重组表达 Recombinant expression	参考文献 References
	PEXC CBH2	*P. expansum* CMP-1	KGO54790.1	—	[17]
	PEXD CBH2	*P. expansum* d1	KGO54790.1	—	[17]
	PITP CBH2	*P. italicum* PHI-1	KGO54790.1	—	[17]
GH7	PCA CBH1	*P. canescens* RN3-11-7	AIL95870	*P. canescens*	[18]
	PDIP CBH1	*P. digitatum* Pd1	EKV07924.1	—	[19]
	PDIG CBH1	*P. digitatum* PHI26	EKV09547.1	—	[19]
	PCH CBH1	*P. chrysogenum* FS010	AAX84833.1	*Saccharomyces cerevisiae*	[10,20]
	PGR CBH1	*P. granulatum* MS861833	AGU16949.1	—	NCBI
	PGL CBH1	*P. glabrum* NA-69	AEL78901.1	—	[10]
	PJA CBH1	*P. janthinellum* Biourge IMET 43733	CAA41780.1	*Escherichia coli* *S. cerevisiae*	[10,21]
	POC CBH1	*P. occitanis*	AAT99321.1	—	[10,22]
	POX1 CBH1-1	*P. oxalicum* 144-2	EPS32984.1	*P. oxalicum*	[13,16]
	POX1 CBH1-2	*P. oxalicum* 144-2	EPS30494.1	—	[16]
	POXF CBH1	*P. oxalicum* F67	ACE60553.1	—	[23]
	POXG CBH1-1	*P. oxalicum* GZ-2	AGW24292.1	—	NCBI
	POXG CBH1-2	*P. oxalicum* GZ-2	AGW24291.1	—	NCBI
	POXM CBH1	*P. oxalicum* M	AEF33951.1	*P. pastoris*	[24]
	PRO CBH1-1	*P. roqueforti* FM164	CDM33480.1	—	[25]
	PRO CBH1-2	*P. roqueforti* FM164	CDM37977.1	—	[25]
	PRU CBH1-1	*P. rubens* Wisconsin 54-1255	CAP85526.1	—	[15,16]
	PRU CBH1-2	*P. rubens* Wisconsin 54-1255	CAP94773.1	—	[15,16]

表 2 已知氨基酸序列的青霉内切葡聚糖酶
Table 2 Endoglucanases with known amino acid sequences from *Penicillium* strains

CAZy 家族 CAZy family	酶名称 Enzyme name	来源菌株 Source strain	GenBank Accession number	重组表达 Recombinant expression	参考文献 References
GH5	PBR EG5	*P. brasilianum* IBT 20888	ACB06750.1	*A. oryzae*	[10,26]
	PCR EG5	*P. crustosum* 601	AHA15977.1	—	NCBI
	POX1 EG5-1	*P. oxalicum* 144-2	EPS25573.1	*S. cerevisiae*	[6,16,27]
	PEC EG5	*P. echinulatum* 9A01S2	ACR82487.1	*P. pastoris*	[10,28]
	PJA EG5	*P. janthinellum* Biourge IMET 43733	CAA61740.1	*S. cerevisiae*	[10,29]
	POX1 EG5-2	*P. oxalicum* 144-2	EPS30243.1	—	[6,16]
	POX1 EG5-3	*P. oxalicum* 144-2	EPS35004.1	*P. pastoris*	[6,16,30]
	POX1 EG5-4	*P. oxalicum* 144-2	EPS28765.1	—	[6,16]
	POX1 EG5-5	*P. oxalicum* 144-2	EPS34262.1	—	[6,16]
	POX1 EG5-6	*P. oxalicum* 144-2	EPS34303.1	—	[6,16]
	PRU EG5-1	*P. rubens* Wisconsin 54-1255	XP_002562753.1	—	[15,16]
	PRU EG5-2	*P. rubens* Wisconsin 54-1255	XP_002557514.1	—	[15,16]
	PRU EG5-3	*P. rubens* Wisconsin 54-1255	XP_002562435.1	—	[15,16]
	PRU EG5-4	*P. rubens* Wisconsin 54-1255	XP_002565826.1	—	[15,16]
	PRU EG5-5	*P. rubens* Wisconsin 54-1255	XP_002568455.1	—	[15,16]
GH7	PDEL EG7	*P. decumbens* L-06	ACJ15337.1	*E. coli*	[31]
	POX1 EG7	*P. oxalicum* 144-2	EPS32968.1	*S. cerevisiae*	[6,16,27]
	POXS EG7-1	*P. oxalicum* SJ1	AGG20187.1	—	NCBI
	POXW EG7	*P. oxalicum* seawater	ACS32299.1	—	NCBI
	POXS EG7-2	*P. oxalicum* SJ1	AGG20186.1	—	NCBI
	POXG EG7	*P. oxalicum* GZ-2	AGW24293.1	—	NCBI
	POXM EG7	*P. oxalicum* M	AEF33952.1	—	NCBI
	POX1S EG7	*P. oxalicum* 1SMS	AEC03713.1	—	[32]
	PC7 EG7	*Penicillium* sp. C7	AEG74551.1	—	NCBI
GH12	PRO EG12	*P. roqueforti* FM164	CDM26511.1	—	[25]
	POX1 EG12-1	*P. oxalicum* 144-2	EPS27942.1	—	[6,16]
	POX1 EG12-2	*P. oxalicum* 144-2	EPS31484.1	—	[6,16]

续表 2
Continue table 2

CAZy 家族 CAZy family	酶名称 Enzyme name	来源菌株 Source strain	GenBank Accession number	重组表达 Recombinant expression	参考文献 References
	POX1 EG12-3	*P. oxalicum* 144-2	EPS34052.1	—	[6,16]
	POXG EG12	*P. oxalicum* GZ-2	AGW24294.1	—	NCBI
	PRU EG12-1	*P. rubens* Wisconsin 54-1255	XP_002560942.1	—	[15,16]
	PRU EG12-2	*P. rubens* Wisconsin 54-1255	XP_002561188.1	—	[15,16]
	PRU EG12-3	*P. rubens* Wisconsin 54-1255	XP_002563257.1	—	[15,16]
GH45	POX1 EG45	*P. oxalicum* 144-2	EPS32967.1	*P. pastoris*	[10,33]
	POXG EG45	*P. oxalicum* GZ-2	AGW24295.1	—	NCBI

表 3　已知氨基酸序列的青霉 β-葡萄糖苷酶

Table 3　Beta-glucosidases with known amino acid sequences from *Penicillium* strains

CAZy 家族 CAZy family	酶名称 Enzyme name	来源菌株 Source strain	GenBank Accession number	重组表达 Recombinant expression	参考文献 References
GH1	PDIP BGL1-1	*P. digitatum* Pd1	EKV06671.1	—	[19]
	PDIP BGL1-2	*P. digitatum* Pd1	EKV17719.1	—	[19]
	PDIP BGL1-3	*P. digitatum* Pd1	EKV09110.1	—	[19]
	PDIG BGL1-1	*P. digitatum* PHI26	EKV10376.1	—	[19]
	PDIG BGL1-2	*P. digitatum* PHI26	EKV05985.1	—	[19]
	PDIG BGL1-3	*P. digitatum* PHI26	EKV08292.1	—	[19]
	POX1 BGL1-1	*P. oxalicum* 144-2	EPS29909.1	—	[6,16]
	POX1 BGL1-2	*P. oxalicum* 144-2	EPS34571.1	—	[6,16]
	POX1 BGL1-3	*P. oxalicum* 144-2	EPS26341.1	*P. pastoris*	[6,16,34]
	POX1 BGL1-4	*P. oxalicum* 144-2	EPS25645.1	*E. coli*	[6,16,35]
	PRO BGL1-1	*P. roqueforti* FM164	CDM27831.1	—	[25]
	PRO BGL1-2	*P. roqueforti* FM164	CDM30526.1	—	[25]
	PRO BGL1-3	*P. roqueforti* FM164	CDM34293.1	—	[25]
	PRO BGL1-4	*P. roqueforti* FM164	CDM34691.1	—	[25]
	PRU BGL1-1	*P. rubens* Wisconsin 54-1255	XP_002558153.1	—	[15,16]
	PRU BGL1-2	*P. rubens* Wisconsin 54-1255	XP_002561682.1	—	[15,16]
	PRU BGL1-3	*P. rubens* Wisconsin 54-1255	XP_002559338.1	—	[15,16]
	PEXC BGL1-1	*P. expansum* CMP-1	KGO72250.1	—	[17]
	PEXC BGL1-2	*P. expansum* CMP-1	KGO66673.1	—	[17]
	PEXC BGL1-3	*P. expansum* CMP-1	KGO59438.1	—	[17]
	PEXC BGL1-4	*P. expansum* CMP-1	KGO45418.1	—	[17]
	PEXC BGL1-5	*P. expansum* CMP-1	KGO40891.1	—	[17]
	PEXC BGL1-6	*P. expansum* CMP-1	KGO36609.1	—	[17]
	PEXM BGL1-1	*P. expansum* MD-8	KGO58887.1	—	[17]
	PEXM BGL1-2	*P. expansum* MD-8	KGO56428.1	—	[17]
	PEXM BGL1-3	*P. expansum* MD-8	KGO55835.1	—	[17]
	PEXM BGL1-4	*P. expansum* MD-8	KGO53074.1	—	[17]
	PEXM BGL1-5	*P. expansum* MD-8	KGO51420.1	—	[17]
	PEXM BGL1-6	*P. expansum* MD-8	KGO51339.1	—	[17]
	PEXD BGL1-1	*P. expansum* d1	KGO42386.1	—	[17]
	PEXD BGL1-2	*P. expansum* d1	KGO41046.1	—	[17]
	PEXD BGL1-3	*P. expansum* d1	KGO40162.1	—	[17]
	PEXD BGL1-4	*P. expansum* d1	KGO39433.1	—	[17]
	PEXD BGL1-5	*P. expansum* d1	KGO36996.1	—	[17]
	PEXD BGL1-6	*P. expansum* d1	KGO36915.1	—	[17]
	PITP BGL1-1	*P. italicum* PHI-1	KGO75047.1	—	[17]
	PITP BGL1-2	*P. italicum* PHI-1	KGO74116.1	—	[17]
GH3	PBR BGL3	*P. brasilianum* IBT 20888	ABP88968.1	*A. oryzae*	[10,36]
	PDE BGL3	*P. decumbens* CICC 40361	ADB82653.1	—	NCBI
	PRU BGL3-1	*P. rubens* Wisconsin 54-1255	XP_002557246.1	—	[15,16]

续表 3

Continue table 3

CAZy 家族 CAZy family	酶名称 Enzyme name	来源菌株 Source strain	GenBank Accession number	重组表达 Recombinant expression	参考文献 References
GH3	PRU BGL3-2	*P. rubens* Wisconsin 54-1255	XP_002557931.1	—	[15,16]
	PRU BGL3-3	*P. rubens* Wisconsin 54-1255	XP_002560677.1	—	[15,16]
	PRU BGL3-4	*P. rubens* Wisconsin 54-1255	XP_002561135.1	—	[15,16]
	PRU BGL3-5	*P. rubens* Wisconsin 54-1255	XP_002562038.1	—	[15,16]
	PRU BGL3-6	*P. rubens* Wisconsin 54-1255	XP_002563511.1	—	[15,16]
	PRU BGL3-7	*P. rubens* Wisconsin 54-1255	XP_002563832.1	—	[15,16]
	PRU BGL3-8	*P. rubens* Wisconsin 54-1255	XP_002564856.1	—	[15,16]
	PRU BGL3-9	*P. rubens* Wisconsin 54-1255	XP_002565451.1	—	[15,16]
	PRU BGL3-10	*P. rubens* Wisconsin 54-1255	XP_002566312.1	—	[15,16]
	PRU BGL3-11	*P. rubens* Wisconsin 54-1255	XP_002568350.1	—	[15,16]
	PRU BGL3-12	*P. rubens* Wisconsin 54-1255	XP_002568817.1	—	[15,16]
	PDIG BGL3-1	*P. digitatum* PHI26	EKV14665.1	—	[19]
	PDIG BGL3-2	*P. digitatum* PHI26	EKV06492.1	—	[19]
	PDIG BGL3-3	*P. digitatum* PHI26	EKV19863.1	—	[19]
	PDIG BGL3-4	*P. digitatum* PHI26	EKV14433.1	—	[19]
	PDIG BGL3-5	*P. digitatum* PHI26	EKV12359.1	—	[19]
	PDIG BGL3-6	*P. digitatum* PHI26	EKV12046.1	—	[19]
	PDIG BGL3-7	*P. digitatum* PHI26	EKV09033.1	—	[19]
	PDIP BGL3-1	*P. digitatum* Pd1	EKV07490.1	—	[19]
	PDIP BGL3-2	*P. digitatum* Pd1	EKV20425.1	—	[19]
	PDIP BGL3-3	*P. digitatum* Pd1	EKV12891.1	—	[19]
	PDIP BGL3-4	*P. digitatum* Pd1	EKV12397.1	—	[19]
	PDIP BGL3-5	*P. digitatum* Pd1	EKV21779.1	—	[19]
	PDIP BGL3-6	*P. digitatum* Pd1	EKV20192.1	—	[19]
	PDIP BGL3-7	*P. digitatum* Pd1	EKV21659.1	—	[19]
	POC BGL3	*P. occitanis*	ABS71124.1	—	NCBI
	POXJ BGL3	*P. oxalicum* JU-A10	ACD86466.1	*T. reesei*	[37,38]
	POX1 BGL3-1	*P. oxalicum* 144-2	EPS26627.1	*P. pastoris*	[6,16,34]
	POX1 BGL3-2	*P. oxalicum* 144-2	EPS27165.1	—	[6,16]
	POX1 BGL3-3	*P. oxalicum* 144-2	EPS27792.1	*P. pastoris*	[6,16,34]
	POX1 BGL3-4	*P. oxalicum* 144-2	EPS27960.1	*P. pastoris*	[6,16,34]
	POX1 BGL3-5	*P. oxalicum* 144-2	EPS28539.1	—	[6,16]
	POX1 BGL3-6	*P. oxalicum* 144-2	EPS29302.1	—	[6,16]
	POX1 BGL3-7	*P. oxalicum* 144-2	EPS34057.1	*P. pastoris*	[6,16,34]
	POXG BGL3	*P. oxalicum* GZ-2	AGW24289.1	—	NCBI
	PRO BGL3-1	*P. roqueforti* FM164	CDM29429.1	—	[25]
	PRO BGL3-2	*P. roqueforti* FM164	CDM32885.1	—	[25]
	PRO BGL3-3	*P. roqueforti* FM164	CDM34537.1	—	[25]
	PRO BGL3-4	*P. roqueforti* FM164	CDM37343.1	—	[25]
	PEXC BGL3-1	*P. expansum* CMP-1	KGO53001.1	—	[17]
	PEXC BGL3-2	*P. expansum* CMP-1	KGO49389.1	—	[17]
	PEXC BGL3-3	*P. expansum* CMP-1	KGO36472.1	—	[17]
	PEXM BGL3-1	*P. expansum* MD-8	KGO61224.1	—	[17]
	PEXM BGL3-2	*P. expansum* MD-8	KGO58976.1	—	[17]
	PEXM BGL3-3	*P. expansum* MD-8	KGO58214.1	—	[17]
	PEXM BGL3-4	*P. expansum* MD-8	KGO52728.1	—	[17]
	PEXM BGL3-5	*P. expansum* MD-8	KGO51054.1	—	[17]
	PEXD BGL3-1	*P. expansum* d1	KGO46978.1	—	[17]
	PEXD BGL3-2	*P. expansum* d1	KGO46245.1	—	[17]
	PEXD BGL3-3	*P. expansum* d1	KGO44213.1	—	[17]
	PEXD BGL3-4	*P. expansum* d1	KGO43702.1	—	[17]
	PEXD BGL3-5	*P. expansum* d1	KGO39841.1	—	[17]
	PITP BGL3-1	*P. italicum* PHI-1	KGO72155.1	—	[17]
	PITP BGL3-2	*P. italicum* PHI-1	KGO64006.1	—	[17]

分析纤维素酶氨基酸序列得知，大部分青霉纤维素酶具有典型的真菌纤维素酶分子结构，通常由催化功能域（catalytic module）、碳水化合物结合功能域（carbohydrate-binding module，CBM）和连接肽（peptide linker）组成。但是，少数青霉纤维素酶没有CBM，例如来自巴西青霉的EGa[11]。根据CAZy数据库分析，青霉可以分泌完整的纤维素酶系，包括GH5、GH7、GH12和GH45家族的EG，GH6和GH7家族的CBH，以及GH1和GH3家族的BGL。迄今，已经公布了15个青霉属菌株的基因组序列，分别是橘灰青霉（*P. aurantiogriseum*）NRRL 62431[39]，沙门柏干酪青霉（*P. camemberti*）FM013[25]，产黄青霉（*P. chrysogenum*）NCPC 10086[40]和KF-25[41]，指状青霉（*P. digitatum*）Pd01-ZJU[42]、Pd1[19]和PHI26[19]，扩展青霉（*P. expansum*）CMP-1[17]、d1[17]和PHI-1[17]，意大利青霉（*P. italicum*）PHI-1[17]，覃青霉（*P. paxilli*）ATCC 26601[NCBI]，草酸青霉（*P. oxalicum*）144-2[16]，娄地青霉（*P. roqueforti*）FM164[25]和产红青霉（*P. rubens*）Wisconsin 54-1255[15]。基因组序列分析表明，产红青霉和草酸青霉分别含有26个（8个EG，3个CBH，15个BGL）和25个（11个EG，3个CBH，11个BGL）纤维素酶基因，是青霉属中已知拥有最丰富的纤维素酶菌种。相比其它属真菌，例如木霉和曲霉，青霉拥有的纤维素酶基因数量多于木霉，略少于曲霉（图1）[16]。虽然曲霉含有纤维素酶基因数量最多，但是纯化或者高效表达曲霉纤维素酶以应用于木质纤维素水解的研究很少。目前，曲霉主要作为生产半纤维素酶和果胶酶的工业菌株。另外，只有研究草酸青霉以用于生物质的酶解报道，对其它青霉菌株的研究主要用于抗生素等工业生产。

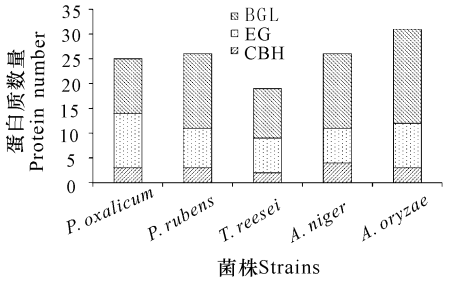

图1　5种真菌中纤维素酶数量的比较[16]

Fig. 1　Comparison of numbers of cellulases among five fungal species[16]

1.2　青霉纤维素酶的进化分析

真菌中，纤维素酶基因并非生命活动的必需基因，即使在亲缘关系较近的物种之间，其数目和种类也存在很大的差异，因此，系统地对青霉纤维素酶进行进化分析很有必要[6]。用系统进化分析软件对纤维素酶进行进化分析（图2）表明：（1）同一个菌株中纤维素酶的近缘关系是不同的，例如，草酸青霉两个CBH1分布在两个亚簇内，POX1 CBH1-1与PCA CBH1和PJA CBH1具有近缘关系；然而，POX1 CBH1-2与PRU CBH1-2，PRO CBH1-1，PDIP CBH1和PDIG CBH1具有近缘关系（图2a）；（2）除了GH5家族EG外，所有的青霉纤维素酶都按照CAZy分类系统进行分簇；部分GH5家族EG，例如POX1 EG5-6，PRU EG5-2等更加接近GH12家族EG（图2b）；（3）每个GH家族都可以分为3～5个亚家族，亲缘关系具有多样性（图2）。

2　调节因子

真菌纤维素酶产量低、成本高是限制工业生产木质纤维素燃料乙醇产业的最大障碍。通过遗传工程改造天然真菌菌株是比较理想的途径，例如，纤维素酶合成调控因子之一转录激活因子在宿主的过量表达，使宿主纤维素酶产量提高[43,44]，因此，清楚了解纤维素酶基因的表达调控机制对提高纤维素酶产量具有重要意义。目前，丝状真菌中纤维素酶合成调控机制研究主要集中在木霉属和曲霉属，代表菌株分别为里氏木霉和黑曲霉。青霉属的研究仍处在初始阶段（图3）。

在丝状真菌中，已知的纤维素酶基因表达的调控因子主要包括转录激活因子、碳源代谢抑制因子、pH值和氮源调控因子、转录诱导因子，以及光信号蛋白、G蛋白信号和cAMP信号通路蛋白[6,9,16,43～45]。纤维素酶合成表达调控具有以下特点：（1）纤维素酶基因在转录水平受转录激活因子和转录阻遏因子共调控；（2）纤维素酶基因在转录水平受外部诱导物的诱导（纤维寡糖及其衍生物、木聚糖、光信号蛋白、G蛋白信号和cAMP信号蛋白）和代谢产物的阻遏（葡萄糖、纤维二糖）；（3）多种纤维素酶基因在转录水平被共调控，例如：ClrB和XlnR共同调控外切葡聚糖酶基因（*celC*，*celD*，*cbhD*）、β-葡萄糖苷酶基因（*bgl5*）；（4）纤维素酶基因合成调控需要多种信号蛋白的共同参与[6,9,16,43～54]。复杂的纤维素酶合成调控是所有调控因子共同作用的结果。目前，已实验证实的青霉属纤维素酶合成调控因子同样遵循以上特点（图3），但是，相比其它丝状真菌，其已知的合成调控网络仍不完整，还需进一步研究和完善。基因组学、转录组学和蛋白组学的应用给全面认识青霉纤维素酶合成调控网络提供了良好平台。草酸青霉144-2基因组注释表明其包含476个转录因子，与里氏木霉的转录

因子数目(484 个)相似,比产红青霉少 108 个转录因子。主要表现在含 Zn_2Cys_6,C_2H_2 锌指、着丝粒蛋白 B 的 DNA 结合域(centromere protein B DNA-binding domain)、CCHC-型锌指结构域的蛋白质。同时,注释基因组也发现了 117 个蛋白激酶、23 个蛋白磷酸酶、33 个 Ras 超家族小 GTP 酶,以及参与 G 蛋白信号通路、cAMP 信号通路、丝裂原活化蛋白激酶通路、钙离子信号通路等信号转导过程的蛋白质[6,16],为大量识别新的调控因子提供了良好资源。

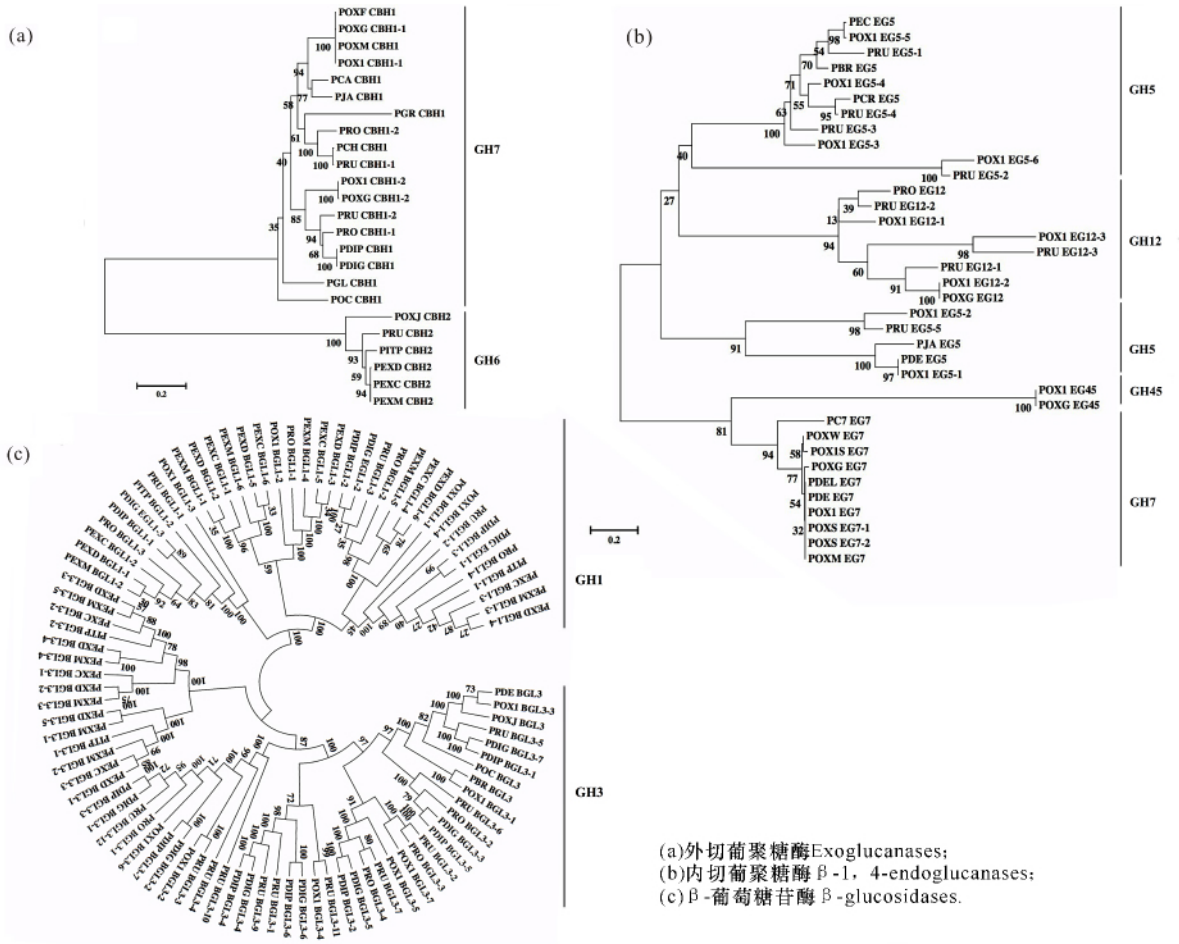

图 2　青霉纤维素酶的系统进化分析

Fig. 2　Phylogenetic analysis of cellulases in *Penicillium*

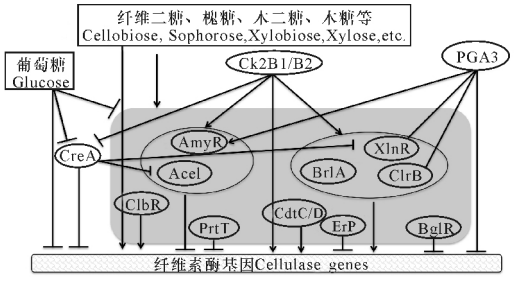

图 3　青霉属纤维素酶合成调控网络[6,9,16,45,47~54]

Fig. 3　Regulatory network of cellulase synthesis in *Penicillium* [6,9,16,45,47~54]

箭头代表激活作用,平端代表阻遏作用;AceI, AmyR, BglR, BrlA, ClbR, ClrB, PrtT, XlnR:转录因子;CdtC/D:纤维糊精转运子;CK2B1/B2:蛋白激酶;CreA:碳代谢阻遏转录因子;Erp:p24γ 蛋白;PGA3:G 蛋白 α 亚基。

Arrow and flat-end represent activation and repression, respectively; AceI, AmyR, BglR, BrlA, ClbR, ClrB, PrtT, XlnR: Transcriptional factors; CdtC/D: Cellodextrin transporters; CK2B1/B2: Protein kinase; CreA: Carbon source-dependent transcriptional repressor; Erp: p24γ protein; PGA3: G protein α subunit.

大部分纤维素酶合成调控因子都属于锌指蛋白转录因子超家族。锌指蛋白是一类具有手指结构域的转录因子,对基因表达调控、细胞分化、胚胎发育等方面具有重要作用,是真核生物中分布最广的一类蛋白[55,56]。锌指蛋白分为 3 大类:(1)经典锌指蛋白 Cys_2His_2 型,通常以单体的形式与核酸相互作用[57],例如碳代谢阻遏调控因子 CreA[47];(2)Cys_4 型,以二聚体的形式与 DNA 相互作用,其异源二聚体与目标基因的正向重复序列相结合,同源二聚体识别反向重复序列[58];(3)$Zn(II)_2Cys_6$ 型,可以单体、同源或异源二聚体形式与 DNA 相结合,是真菌所特有的转录因子[55,56,58,59],例如 XlnR[60]。研究证明,尽管属于同一个家族,不同转录因子的调控分子机制也不尽相同[45]。

丝状真菌中,研究比较深入的是转录激活因子

XlnR(图 4):(1)XlnR 的 N 端包含有一个锌指双核簇($Zn(II)_2Cys_6$)和一个推测的卷曲螺旋区;(2)XlnR 的 C 端包含一个 D-葡萄糖抑制区和一个自我修饰区;(3)$XlnR_{635\sim668}$参与蛋白进入细胞核过程;(4)$XlnR_{1\sim668}$决定木聚糖酶的产量;(5)XlnR 的调节作用有可能是由 D-木糖诱导的磷酸化引起的[46,60]。但是,准确的、真实的调控分子机理还需要进一步研究和证实。

最新研究表明,在丝状真菌中,XlnR 在不同的菌株中具有激活主要木聚糖酶基因转录的功能,但是,在纤维素酶或半纤维素酶基因的转录激活方面,依据宿主的不同而不同[61]。因此,青霉属 XlnR 的详细调控机制仍需更深入的研究。

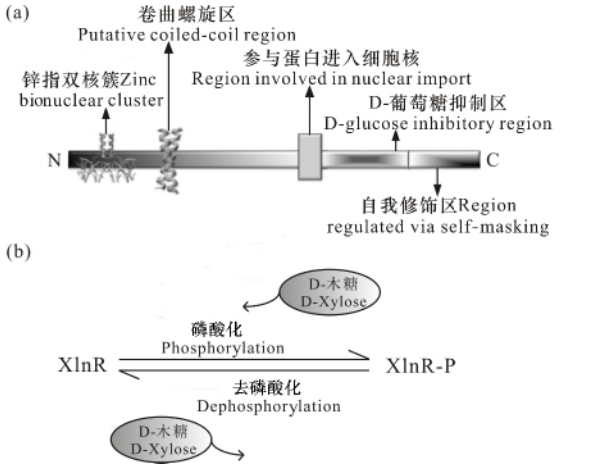

图 4 转录调节因子 XlnR 结构域组成(a)以及推测的调节分子机制(b)[46,60]

Fig. 4 Schematic illustration of domain organization of transcriptional factor XlnR (a) and its proposed regulatory mechanism at molecular level (b)[46,60]

3 青霉纤维素酶基因重组表达

为提高纤维素酶产量、降低纤维素酶成本,除了深入研究纤维素酶合成调控外,建立优良的纤维素酶基因重组表达系统,高效表达基因是最经济、最快速的途径。多年来,纤维素酶基因的表达系统主要集中在:(1)细菌原核表达系统——革兰氏阴性菌:如大肠杆菌(*E. coli*) BL21 或 Rosetta,运动发酵单胞菌(*Zymomonas mobilis*);革兰氏阳性菌:如梭菌(*Clostridium*)等;(2)酵母表达系统:如酿酒酵母(*S. cerevisiae*)和毕赤酵母(*P. pastoris*)等;(3)丝状真菌表达系统:如黑曲霉、里氏木霉等[62]。

3.1 细菌原核表达系统

细菌原核表达系统是目前最成熟的表达系统,已用该表达系统成功表达了 100 多种纤维素酶和木聚糖酶基因。根据细胞壁的不同,革兰氏阳性菌利用双精氨酸分泌途径(Twin arginine translocation pathway,Tat)、丝氨酸分泌途径(Sec-protein translocation pathway)等转运表达的纤维素酶到胞外;革兰氏阴性菌由于有外部膜限制胞内转运蛋白的运输,需要利用多种分泌途径,如Ⅰ~Ⅶ型分泌系统。大肠杆菌是研究最多的宿主,蛋白表达量一般为 11.2~90.0 mg/L[62,63]。枯草芽胞杆菌(*Bacillus subtilis*)和运动发酵单胞菌主要应用在表达细菌产纤维素酶领域。仅有 3 个分别来自微紫青霉、斜卧青霉和草酸青霉的纤维素酶基因 PJA CBH1、PDE EG7 和 POX1 BGL1-4 在大肠杆菌中成功表达(表 1~表 3),但是表达过程中,发现胞外分泌量低、形成包涵体等问题。因此,细菌原核表达系统表达真菌蛋白有着天然的缺陷,如胞外分泌量低,缺乏蛋白质翻译后的修饰功能(糖基化、磷酸化等),易形成包涵体,以及表达的蛋白质无酶活等[6]。

3.2 酵母表达系统

酵母菌是公认的安全菌株,最早发展用于异源蛋白表达的真核表达系统。酵母菌表达、分泌纤维素酶主要经过 3 个步骤:(1)纤维素酶基因在内质网(endoplasmic reticulum,ER)翻译表达;(2)转移到高尔基体(Golgi apparatus)进行后加工;(3)最后通过分泌小囊(secretory vesicles)分泌到胞外,蛋白表达量大约为 1000 mg/L[62,63]。目前,实验证实多种酵母菌可以表达纤维素酶基因,包括酿酒酵母[64]、毕赤酵母[65,66]、巴斯德酵母(*S. pastorianus*)[67]、马克斯克鲁维酵母(*Kluyveromyces marxiaus*)[68]。迄今,共有 14 个青霉纤维素酶基因在酵母表达系统成功表达,包括酿酒酵母和毕赤酵母(表 1~表 3)。结果显示,酵母表达系统存在酶胞外分泌量低、过度糖基化、翻译后修饰少,不能很好地表达原始纤维素酶的功能等问题。

3.3 丝状真菌表达系统

丝状真菌表达系统应用于纤维素酶基因的表达起步较晚,但是发展非常迅速。自从 1997 年第一个来源于里氏木霉的内切葡聚糖酶基因 *egl2* 在米曲霉成功表达[69]以来,大量的纤维素酶基因在黑曲霉、米曲霉、里氏木霉中表达。丝状真菌表达系统弥补了原核表达系统和酵母表达系统的缺陷,具有蛋白分泌强,能对真核来源的蛋白进行正确的折叠和修饰,表达的蛋白活性高等优点,其表达蛋白量为 14000~19000 mg/L[6,62,63]。已有 6 个青霉纤维素酶基因成功在丝状真菌中表达,所用宿主包括里氏木霉、草酸青霉、米曲霉和变灰青霉(表 1~表 3)。但是该系统存在转化效率低,内源纤维素酶基因本源表达等

问题。

对于青霉纤维素酶基因，仅有不到20%的纤维素酶基因(表1～表3)得到了表达，但存在表达量低等问题，离工业生产要求相差甚远。因此，为更大程度地利用青霉纤维素酶基因资源，构建一个高效的、稳定的丝状真菌外源基因表达系统非常重要[70]，可通过选用强启动子、敲除蛋白酶基因、液泡蛋白分选基因(vacuolar protein sorting gene)等提高外源基因的表达量和表达产物的胞外分泌量。

4 展望

随着石油等不可再生能源的枯竭，非粮生物质能源产业的蓬勃发展是必然趋势。其中，木质纤维素燃料乙醇产业将发挥其经济价值。目前，仅靠里氏木霉作为工业生产纤维素酶菌株是很难满足大规模工业生产的需求的，况且该菌株天然存在胞外BGL活力低的缺陷。因此，应该以分泌完整纤维素酶系的青霉属菌株作为另外一种选择，深入研究青霉属菌株的纤维素酶基因的合成调控因子，通过操纵合成调控因子来提高纤维素酶产量，或构建高效的丝状真菌分泌表达系统，实现纤维素酶的产量和酶活性的提高，降低生产成本，满足工业生产需求。

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