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专论与综述

黄曲霉菌主要真菌毒素次级代谢与调控的研究进展

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摘　要：黄曲霉菌(*Aspergillus flavus*)是一种腐生型好氧真菌，其次级代谢产生的黄曲霉毒素(Aflatoxin，AFT)是一种强致癌性剧毒物质。黄曲霉菌侵染农作物导致相关农产品黄曲霉毒素的污染，危及食品安全及人和动物的健康。黄曲霉菌有 8 条染色体，基因组大小约 37 Mb，含有 13 000 多个功能基因，55 个次级代谢基因簇，其中只明确了 AFT、环匹阿尼酸(Cyclopiazonic acid，CPA)和黄曲霉震颤素(Aflatrem) 3 个次级代谢基因簇的特征。次级代谢基因簇的表达受不同环境条件、次级代谢调控因子、酶活性、复杂的脂氧合物转导信号及群体密度效应的调控。LaeA 和 VeA 是抑制 AFT、CPA 和黄曲霉震颤素等真菌毒素生物合成的次级代谢调控因子，抑制加氧酶类(Ppo 和 Lox)的表达则能促进真菌毒素的合成，而其氧化产物(脂氧合物)则是真菌-寄主互作的重要信号分子。群体密度和水解酶类也影响黄曲霉菌的次级代谢，群体密度高能降低黄曲霉毒素的生成量而增加分生孢子的形成；α-淀粉酶、果胶酶、蛋白酶等酶活性的改变可以影响黄曲霉菌分生孢子萌发、菌丝生长，以及真菌毒素的次级代谢。本文系统评述了黄曲霉主要真菌毒素的次级代谢与调控的研究进展。此外，对黄曲霉次级代谢物的研究也做了进一步的评述和讨论。

关键词：黄曲霉菌，真菌毒素，次级代谢，调控

Progresses on research of secondary metabolite and regulation of primary mycotoxins in *Aspergillus flavus*

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Abstract: Aflatoxin (AFT) contamination caused by *Aspergillus flavus*, a saprophytic aerobic fungus, in several crops such as peanut, maize and cottonseed is considered as the most serious factor influencing food safety concerning animal and human health worldwide. Whole genome sequences of *A. flavus* have been released and the genome size is about 37 Mb on 8 chromosomes encoding over 13 000 functional genes. In the whole genome sequences of *A. flavus* (NRRL3357), 55 putative secondary metabolite clusters have been identified based on backbone enzyme gene analysis by SMURF, and only three secondry metabolite clusters including AFT, cyclopiazonic acid (CPA) and aflatrem have been characterized. The above three secondary metabolite clusters are regulated by

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different environmental factors, secondary metabolite regulators, enzymatic activity, oxylipin and quorum sensing. AFT, CPA and aflatrem biosynthesis are inhibited by the global regulators, LaeA and VeA, in secondary metabolism. A family of oxylipin-producing oxygenases and their products encoded by *ppo* and *lox* can regulate sclerotia and conidia production and secondary metabolism in *A. flavus*. The profile of secondary metabolites could be influenced by variation of fungi density because higher fungi density will induce higher sporulation with decreased AFT. Most strains of *A. flavus* can produce numerous hydrolytic enzymes including α-amylases, pectinases, proteases, and lipases, which are believed to be important for fungal infection and virulence to host tissue. Further research on regulation of secondary metabolism and mycotoxins produced in *A. flavus* are also discussed.

Keywords: *Aspergillus flavus*, Mycotoxins, Secondary metabolite, Regulation

黄曲霉菌(*Aspergillus flavus*)是子囊菌亚门(Ascomycotina)、曲霉属(*Aspergillus*)一种常见的腐生型好氧真菌(http://www.aspergillusflavus.org/index.html)，主要分布在 16°–35°的温带地区，而在高于 45°纬度的地区则极少见[1]。黄曲霉菌在土壤中以分生孢子或菌核的形式存在，在植物组织中则以菌丝体的形式存在。菌核能在极端环境条件下(高温、干旱等)存活，并能产生分生孢子或子囊孢子[2-3]。

黄曲霉菌是人和动、植物的共同病原菌。人和动物长期接触黄曲霉菌和其他 20 多种曲霉菌可诱发哮喘、外源性肺泡炎和过敏性支气管曲霉病等疾病[4-6]。在作物田间生长期间，即作物收获前，黄曲霉菌能诱发多种农作物病害的发生，如玉米穗腐病、花生曲霉病、棉花棉铃曲霉病等病害的发生[1,7]。

黄曲霉毒素(Aflatoxins，AFT)[1]、环匹阿尼酸(Cyclopiazonic acid，CPA)、黄曲霉震颤素(Aflatrem)[8]等次级代谢物是全球范围内危及食品安全和人类健康的主要因素之一。黄曲霉菌侵染农作物后可导致农产品黄曲霉毒素污染的发生。黄曲霉菌侵染并不一定导致作物显著减产，但是黄曲霉毒素污染可造成巨大的经济损失。黄曲霉毒素是一类具有相似分子结构和理化性质的化合物(图 1)，也是迄今发现的理化性质最稳定的真菌毒素。1993 年黄曲霉毒素被世界卫生组织(WHO)的国际癌症研究机构(IARC)划定为Ⅰ级致癌物，是一种毒性极强的物质[9]。据联合国粮食及农业组织(Food and Agriculture Organization，FAO)报道[7]，全球每年约有 25%的农作物遭受霉菌及其毒素的污染，其中约 2%的农产品因毒素污染超标而失去利用价值。在美国，每年真菌毒素污染造成的平均经济损失约为 10 亿美元，其中黄曲霉毒素污染造成的损失占很大比例[10]。尽管亚洲和非洲等发展中国家的黄曲霉毒素污染所造成的经济损失没有准确的统计数据，但应该比美国严重得多[8]，由此估计全球每年因毒素污染而造成的直接或间接经济损失达数百亿美元。人类和动物食用被黄曲霉毒素污染的食物，可致畸、致突变、致癌，甚至导致死亡[11-18]。印度和肯尼亚等国家均发生过多起因黄曲霉毒素急性中毒一次性导致数百人死亡的恶性食品安全事件[13]。

随着分子生物学新理论和新技术的发展，尤其是基因组学和生物信息学等研究的快速发展，曲霉菌的研究、防控与应用的深度和广度进一步拓展。黄曲霉毒素、环匹阿尼酸、黄曲霉震颤素等有毒次级代谢物的生物合成都被重点研究，尤其是黄曲霉毒素的生物合成途径已得到较为全面的阐释[19-24]。本文拟评述黄曲霉菌基因组学研究概况、有毒次级代谢物的生物合成与调控的研究进展，旨在为深化黄曲霉有毒物质次级代谢分子机制及其寄主抗性遗传改良的研究提供参考。

1 黄曲霉基因组学研究概况

2003 年 6 月，“真菌基因组计划”选择了 44 种真菌开展全基因组测序，优先开展了与植物和人类致病菌相关的 10 个属，即青霉(*Penicillium*)、曲霉(*Aspergillus*)、组织胞浆菌(*Histoplasma*)、球孢子菌(*Coccidioides*)、镰刀菌(*Fusarium*)、脉孢菌

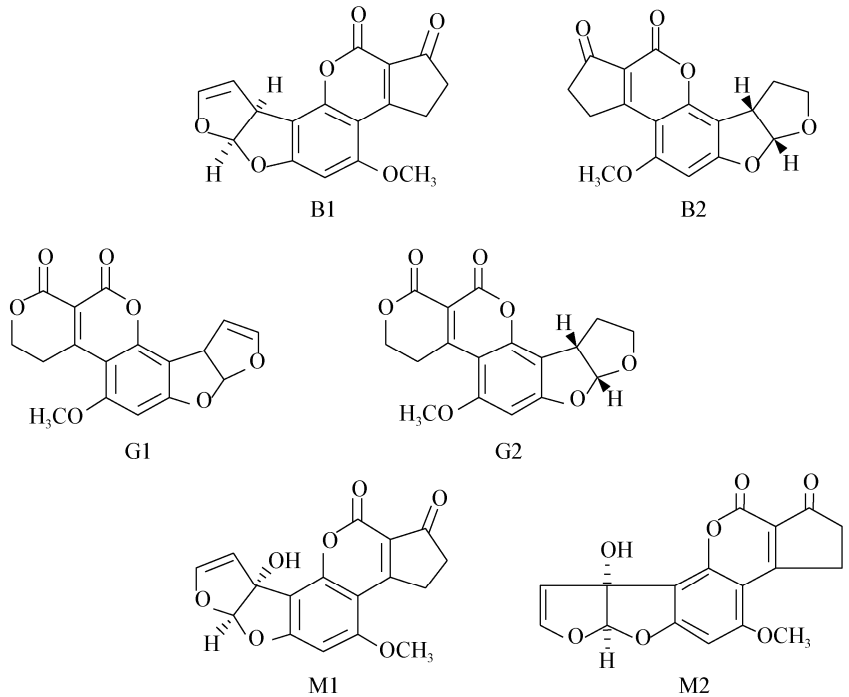

图 1　黄曲霉毒素化学结构式

Figure 1　Chemical structural formula of the primary aflatoxin

注：黄曲霉毒素化学结构式均来源于 SIGMA 网站(http://www.sigmaaldrich.com/china-mainlan d.html)(2013-12-30).

Note: The chemical structural formula of the primary aflatoxin derived from the SIGMA web site (http://www.sigmaaldrich.com/china-mainland.html) (2013-12-30).

(*Neurospora*)、假丝酵母(*Candida*)、裂殖酵母(*Schizosaccharomyces*)、隐球酵母(*Cryptococcus*)和锈病菌(*Puccinia*)属真菌的测序。自 2004 年公布构巢曲霉(*Aspergillus nidulans*)基因组信息开始，研究者们至今已完成米曲霉、黄曲霉、构巢曲霉、烟曲霉、土曲霉、黑曲霉、棒曲霉、酱油曲霉和白曲霉 9 种曲霉属真菌基因组的测序(表 1)。尽管所有曲霉属真菌都有 8 条染色体，但基因组大小存在差异(表 1)[25-26]。2009 年，J. Craig Venter 研究所公布了黄曲霉菌(*A. flavus*)的全基因组序列信息，基因组大小约 37 Mb，含有 13 000 多个功能基因[27-28]。串联基因的额外复制导致黄曲霉菌和米曲霉菌的基因组较大，如烟曲霉(约 30 Mb)、土曲霉(30 Mb)和黑曲霉(34 Mb)等均比黄曲霉菌和米曲霉菌的小[29-30]。

随着研究的发展和深入，相关网站(http://www.aspergillusflavus.org/genomics/，NCBI，http://www.fgsc.net/aspergillus/asperghome 等)对黄曲霉菌基因组序列信息予以更新[31]。黄曲霉菌基因组信息为真菌功能基因组学的研究提供了平台，为有害次级代谢物合成机制的阐释、新抗菌药物的研发、作物抗性遗传改良、黄曲霉菌相关疾病(病害)的预防和治疗，以及黄曲霉毒素污染的防控提出了新战略方向。有趣的是，黄曲霉是一种缺乏 DNA 甲基化的物种[32]，其他已发现缺乏 DNA 甲基化的物种还有果蝇(*Drosophila melanogaster*)、面粉甲虫(*Tribolium castaneu*)、酿酒酵母(*Saccharomyces cerevisiae*)、粟酒裂殖酵母(*Schizosaccharomyces pombe*)和秀丽隐杆线虫(*Caenorbabditis elegans*)[33]。

基因组测序研究表明，黄曲霉菌和米曲霉菌是同一种真菌，米曲霉菌是黄曲霉菌人工驯化得到的一个亚种[34]。大部分丝状真菌的端粒重复序列是 TTAGGG，而黄曲霉菌和米曲霉菌具有相同的特异性端粒重复序列(TTAGGGTCAACA)[35-36]。但是，黄曲霉菌和米曲霉菌的 ITS 序列具有特异性，

表 1 曲霉属真菌全基因组测序基本信息
Table 1 Whole genome sequence information of some species in genus *Aspergillus*

物种 Species	测序菌株 Isolate for sequencing	基因组大小 Genome size (Mb)	GC percent (%)	基因数目 Number of genes	蛋白数目 Number of proteins
黄曲霉 *Aspergillus flavus*	NRRL3357	36.89	48.4	13 485	13 485
构巢曲霉 *Aspergillus nidulans*	FGSC A4	29.83	50.4	10 597	10 534
米曲霉 *Aspergillus oryzae*	RIB40	37.12	48.3	25 272	24 880
米曲霉 *Aspergillus oryzae*	3.042	36.58	48.3	11 640	11 397
烟曲霉 *Aspergillus fumigatus*	Af293	29.39	49.8	9 969	9 650
烟曲霉 *Aspergillus fumigatus*	A1163	29.21	49.5	10 176	9 948
烟曲霉 *Aspergillus fumigatus*	AF210	28.90	49.3	53	20
土曲霉 *Aspergillus terrus*	NIH2624	29.36	52.9	10 551	10 401
黑曲霉 *Aspergillus niger*	CBS513.88	34.01	50.4	11 408	11 182
黑曲霉 *Aspergillus niger*	ATCC1015	34.85	50.3	10 947	10 950
棒曲霉 *Aspergillus clavatus*	NRRL1	27.86	49.2	9 379	9 121
酱油曲霉 *Aspergillus sojae*	NBRC4239	39.77	48.1	–	–
白曲霉 *Aspergillus kawachii*	IFO4308	37.11	49	11 488	11 491

注：表中曲霉属真菌基因组测序信息均来源于 NCBI 网站(http://www.ncbi.nlm.nih.gov/genome/)(2013-10-11).

Note: *Aspergillus* genome sequencing information in Table 1 is derived from the NCBI website (http://www.ncbi.nlm.nih.gov/genome/) (2013-10-11).

可以作为有效区分这两种曲霉菌的标记[37]。黄曲霉菌和米曲霉菌的比较基因组学研究发现，黄曲霉菌仅有 306 个特异基因，米曲霉菌的特异基因有 332 个，而这些差异基因的功能目前还不明确[34]。尽管这两种曲霉菌的大部分次级代谢基因簇是相同的，但是黄曲霉菌能产生 AFT 和 CPA 等多种有毒的次级代谢物，而米曲霉菌则是安全的食品微生物，不会产生对人类有毒有害的次级代谢物，因而在食品发酵工业中具有广泛应用[38]。

2 次级代谢基因簇

一般来说，次级代谢物是生物生长到一定阶段才产生的化学结构复杂、对该生物无明显生理功能或并非是该生物生长和繁殖所必需，但对其它生物存在明显生理活性的物质，如生物碱、毒素、抗生素、色素等。这些生物活性物质对人类或是有害(如黄曲霉毒素)或是有利(如洛伐他丁)。不同生物产生的次级代谢产物不尽相同，有的积累在生物细胞内，有的则分泌到外界环境中。研究表明，在实验室培养条件下，次级代谢产物几乎不会影响真菌的生长和繁殖[38]，但在自然环境中，真菌的次级代谢物具有防御(如防 UV、防捕食)的作用[39]。

次级代谢基因在基因组中通常成簇分布，每一个次级代谢基因簇包含次级代谢物合成所需酶的编码基因和转录调控因子，一个次级代谢基因簇能单独调控该次级代谢途径[40]。黄曲霉菌基因组包括 25 个聚酮合成酶(PKS)基因、3 个类聚酮合成酶(类聚酮合成酶不包含 PKS 所包含的所有典型结构域)基因、18 个非核糖体多肽合成酶(NRPS)基因、14 个类非核糖体多肽合成酶(类非核糖体多肽合成酶不包含 NRPS 包含的所有典型结构域)基因、2 个 PKS-NRPS 基因和 8 个异戊烯基转移酶基因。这些关键酶与其他酶类共同作用合成聚酮化合物(PKS)、非核糖体多肽(NRPS)、PKS-NRPS 杂合物和吲哚生物碱(二甲基丙烯基转移酶，DMATS)等次级代谢物[41-42]。PKS、NRPS 等合成的关键酶对次级代谢基因簇的表达及其表达水平起主要调控作用。黄曲霉菌中存在 55 个次级代谢基因簇(表 2)，但其中只鉴定并明确了 AFT、CPA 和黄曲霉震颤素 3 个次级代谢基因簇及其特征[8]。

表 2 黄曲霉菌的次级代谢基因簇[34]

Table 2 Secondary metabolite clusters in *Aspergillus flavus*[34]

基因簇编号 Cluster number	定位 Location	关键酶类 Backbone enzyme	基因数目 Decorating genes	LaeA 调控 LaeA regulation	次级代谢物 Secondary metabolites
1	IV R	PKS	9	Yes	
2	IV R	DMAT	8	No	
3	IV R	NRPS	1	NA	
4	IV R	NRPS	4	Yes	
5	IV R	PKS	3	Yes	
6	VIII L	NRPS	12	Yes	
7	VIII L	NRPS-like，PKS-like	10	Yes	
8	VIII L	2NRPSs，PKS	9	No	
9	VIII L	2NRPSs，铁载体	10	No	
10	IV L	Arp1 分生孢子色素	3	No	色素
11	II R	NRPS-like	11	Yes	
12	II R	NRPS-like	3	Yes	
13	VII L	NRPS	4	No	
14	VII L	IroE-like 铁载体	2	No	
15	VII L	DMAT	12	Yes	
16	VII L	sidA/sidR，铁载体	1	No	
17	I L	PKS，2PKS-like	9	No	
18	I L	NRPS-like	6	No	
19	V L	DMAT	6	Yes	
20	V L	2PKS	10	No	
21	VI R	2NRPS，ETP-like	36	Yes	木霉素
22	VI R	NRPS	6	Yes	
23	VI R	PKS-NRPS，PKS	17	Yes	
24	VI R	Pes1 PKS	3	Yes	
25	VI R	NRPS-like	8	Yes	青霉素
26	I R	PKS-like，2NRPSs-like	12	No	
27	I R	PKS	5	Yes	
28	I R	NRPS-like	5	No	
29	I R	DMAT	2	No	
30	V R	DMAT，NRPS	6	Yes	
31	V R	NRPS-like	14	Yes	
32	V R	GGPP	19	Yes	黄曲霉震颤素
33	V R	2NRPSs-like，PKS	8	No	
34	VI L	NRPS	8	No	
35	VI L	NRPS-like	5	Yes	
36	III L	2PKS-like	7	No	
37	III L	NRPS-like	3	No	
38	III L	PKS	3	No	
39	III L	PKS	5	No	
40	III L	PKS	9	No	
41	VII R	PKS	3	No	
42	VIII R	PKS	11	No	
43	VIII R	PKS-like，DMAT	18	Yes	

(待续)

(续表)

44	VIII R	PKS	5	No	
45	VIII R	NRPS-like	16	Yes	
46	VIII R	2PKS	4	No	
47	VIII R	NRPS-like	5	No	
48	VIII R	NRPS-like	19	Yes	
49	II L	2PKS-like	6	No	
50	II L	PKS	7	Yes	
51	II L	PKS	5	No	
52	II L	PKS	7	Yes	
53	III R	NRPS	8	No	
54	III R	PKS	30	Yes	黄曲霉毒素
55	III R	DMAT，PKS-NRPS	4	Yes	CPA

注：LaeA 调控是指至少对一个次级代谢基因簇中的两个基因的表达具有上调或下调的作用. NA：暂时还没有获得可利用的分析数据.

Note: LaeA regulation is indicated based on up- or downregulated at least two genes in the same cluster by LaeA in microarrays. NA: No data available.

2.1 黄曲霉毒素代谢基因簇

黄曲霉毒素是一类聚酮衍生物，调控其合成的是第 54 号次级代谢基因簇，位于黄曲霉菌 3 号染色体端粒附近的一个次级代谢基因簇(图 2)[43]。黄曲霉毒素合成的起始物质是乙酰辅酶 A (乙酰 COA，Acetyl-CoA)和丙酰辅酶 A (丙酰 COA，Propionyl-CoA)，起始物质在脂肪酸合成酶(Fasα，Fasβ)的作用下形成己酸盐(Acetate)[44-45]。降散盘衣酸(Norsolorinic acid，NOR)是黄曲霉毒素合成的第一种稳定的前体物质[46]，可进一步转化形成杂色曲霉素(Sterigmatocystin，ST)和黄曲霉毒素(Aflatoxin，AFT)。NOR 的合成必需依赖于黄曲霉毒素基因簇中 *aflA* 基因、*aflB* 基因(脂肪酸合成酶基因)[47]和 *aflC* 基因(聚酮合酶基因)[48]。虽然黄曲霉毒素次级代谢基因簇具有保守性，但在 *A. parasiticus*[49]、*A. flavus*、*A. oryzae*[50]和 *A. sojae*[51]等真菌中存在不同程度的变异，其中只有曲霉属真菌代谢产生黄曲霉毒素或相关的杂色曲霉素。

aflR 基因编码的 Zn(Ⅱ) 2Cys6 型转录因子识别并结合黄曲霉毒素/杂色曲霉素生物合成基因的启动子序列(5′-TCGN$_5$CGR-3′)，调控黄曲霉毒素/杂色曲霉素生物合成基因的表达[52-53]。*aflR* 基因过量表达可增加黄曲霉毒素的合成量，提高毒素生物合成途径其他基因的表达水平，且 *aflR* 基因突变体的黄曲霉毒素/杂色曲霉素生物合成基因不表达，检测不到黄曲霉毒素/杂色曲霉素等产物[52,54-55]。*aflS* 基因编码产物，通过激活 AflR 来调控黄曲霉菌和寄生曲霉菌的黄曲霉毒素合成[56]。

黄曲霉毒素基因簇存在大片段缺失、基因簇重排和点突变等多种变异形式[57-59]。虽然米曲霉(*A. oryzae*)和酱油曲霉(*A. sojae*)等常用食品发酵菌种也有黄曲霉毒素次级代谢基因簇，但 *A. oryzae* 和 *A. sojae* 没有黄曲霉毒素的合成[60]。*A. oryzae* (RIB strains) *aflR* 基因缺失[50]，且 *A. sojae* (Strain 477) *aflR* 和(或) *aflS* 缺失[51]。这些表明，*aflR* 和(或) *aflS* 的缺失或无义突变，可能是 *A. oryzae* 和 *A. sojae* 不能合成黄曲霉毒素的原因。

2.2 环匹阿尼酸代谢基因簇

环匹阿尼酸(Cyclopiazonic acid，CPA)是一种吲哚衍物(Indole-derived ergot alkaloids)(图 3)，是曲霉属和青霉属的几种真菌次级代谢产生的一种真菌毒素[61]，是骨骼肌质浆网 Ca^{2+}-ATP 酶的专化性抑制剂[62]。CPA 基因簇大小 24 kb，包含有编码 PKS-NRPS 杂合物、吲哚生物碱(二甲基丙烯基转移酶，Dimethylallyl tryptophan synthase，DMAT)、单胺氧化酶(Monoamine oxidase)和转录因子的 4 个基因，位于黄曲霉菌 3 号染色体的端粒端，与黄曲霉毒素基因簇相距约 100 kb (图 4)[61,63]。DMAT、单胺氧化酶是黄曲霉菌合成 CPA 的必需因子，PKS-NRPS 在 CPA 的合成过程中也具有重要作用[63-65]。

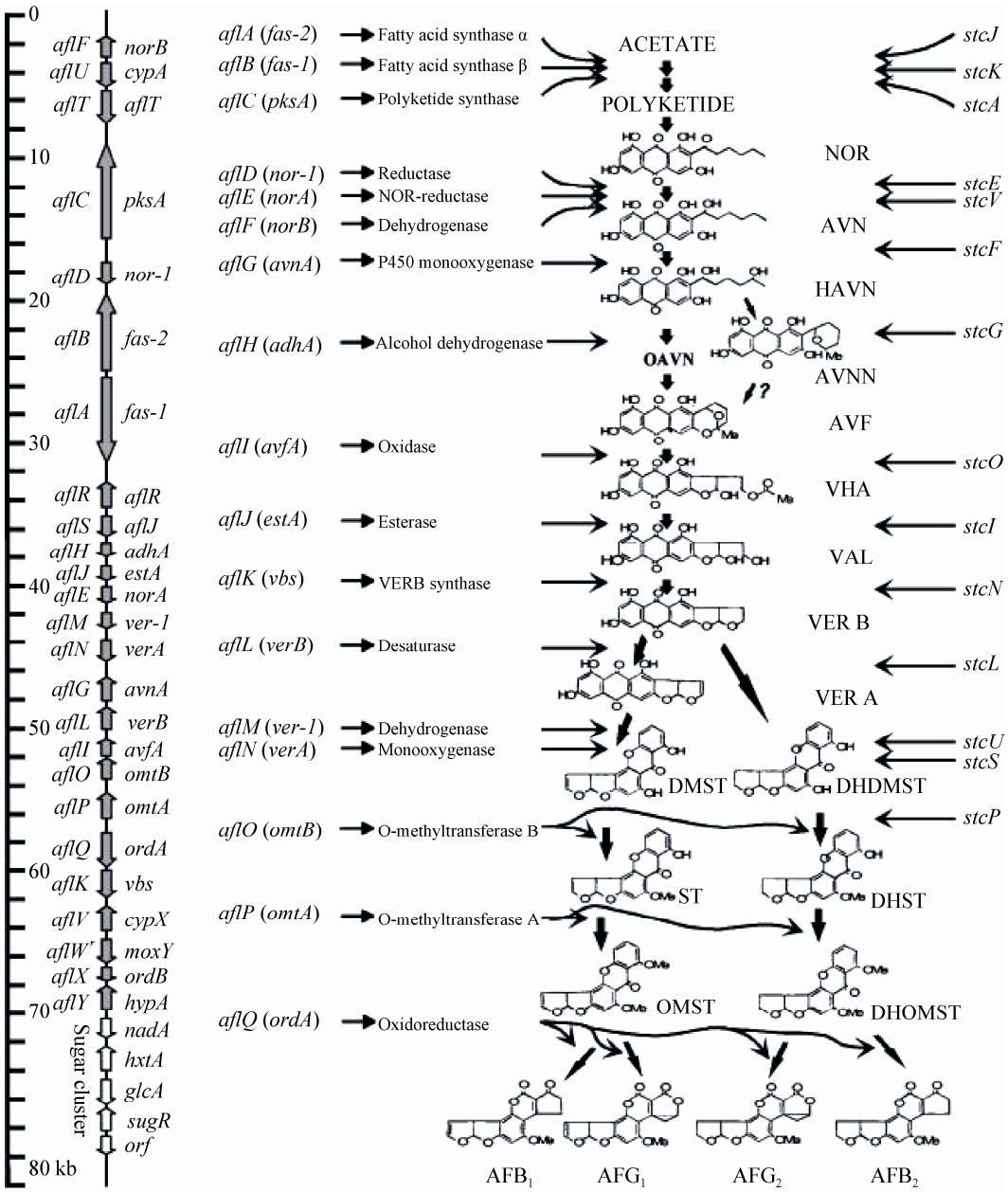

图 2　黄曲霉菌黄曲霉毒素代谢基因簇和黄曲霉毒素合成途径[23]

Figure 2　Clustered genes and the aflatoxin biosynthetic pathway[23]

注：黄曲霉菌的黄曲霉毒素代谢基因簇约含有 30 个不同基因，位于 3 号染色体的端粒端，与 CPA 基因簇间隔较近. 每个基因的新名称标识在图例的左侧，旧名称标识在右侧.

Note: The aflatoxin cluster is composed of approximately 30 different genes and is located near the telomere of chromosome 3, the telomere distal from the CPA cluster. The new names for each gene are shown on the left and the old names on the right. NOR: Norsolorinic acid; AVN: Averantin; HAVN: 5-Hydroxyaverantin; OAVN: Oxoaverantin; AVNN: Averufanin; AVF: Averufin; VHA: Versiconal hemiacetal acetate; VAL: Versiconal; VERB: Versicolorin B; VERA: Versicolorin A; DMST: Demethylsterigmatocystin; DHDMST: Dihydrodemethylsterigmatocystin; ST: Sterigmatocystin; DHST: Dihydrosterigmatocystin; OMST: O-Methylsterigmatocystin; DHOMST: Dihydro-O-methylsterigmatocystin; AFB1: Aflatoxin B1; AFB2: Aflatoxin B2; AFG1: Aflatoxin G1; AFG2: Aflatoxin G2.

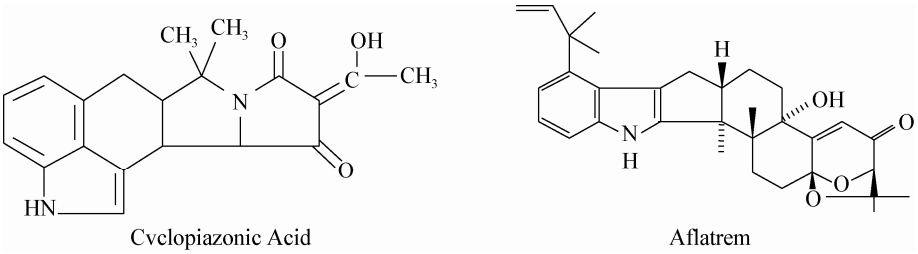

图 3 环匹阿尼酸和黄曲霉震颤素的化学结构式

Figure 3 Chemical structural formula of the cyclopiazonic acid and aflatrem

注：环匹阿尼酸和黄曲霉震颤素化学结构式来源于 SIGMA 网站(http://www.sigmaaldrich.com/china-mainland.html)(2013-12-30).

Note: The chemical structural formula of cyclopiazonic acid and aflatrem derived from the SIGMA web site (http://www.sigmaaldrich.com/china-mainland.html)(2013-12-30).

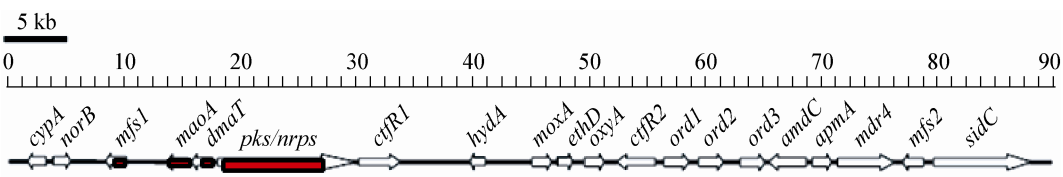

图 4 环匹阿尼酸次级代谢基因簇[63]

Figure 4 Schematic representation of the CPA gene cluster in *A. flavus* NRRL 3357[63]

注：CPA 基因簇与 AFT 基因簇相邻，其中 *norB* 是 AFT 基因簇中与 CPA 基因簇相邻的基因. 红色标识的基因为环匹阿尼酸基因簇的基因.

Note: The CPA cluster is located telomere proximal from the aflatoxin cluster, and the *norB* gene marks the end of the aflatoxin gene cluster. Proposed cyclopiazonic acid biosynthesis genes are shown in red.

2.3 黄曲霉震颤素代谢基因簇

黄曲霉震颤素(Aflatrem)是由曲霉(*Aspergillus*)和青霉(*Penicillium*)次级代谢产生的一种吲哚二萜类真菌毒素(图 3)，具有致肿瘤特性[66-67]。黄曲霉震颤素合成基因簇位于两个不相邻的基因座(*ATM1* 和 *ATM2*)[68]，但不同曲霉菌的黄曲霉震颤素基因簇的结构存在一定的差异(图 5)。其中 *ATM1* 基因簇位于 5 号染色体的端粒区，包含 *atmG*、*atmC* 和 *atmM* 三个基因；*ATM2* 基因簇位于 7 号染色体上，包含 *atmD*、*atmQ*、*atmB*、*atmA* 和 *atmP* 五个基因(图 5)。*atmG* 编码产生的牛儿基二磷酸(Geranylgeranyldiphosphate，GGPP)是四环二萜的核心结构[34]。Paspaline 是黄曲霉震颤素的前体物质，化学性质稳定，*atmC* 编码的异戊烯转移酶与 AtmG、AtmB 是 Paspaline 合成的必需物质。

3 主要有毒次级代谢物合成的调控

3.1 次级代谢全局性调控因子

LaeA 蛋白是曲霉属真菌和其他丝状真菌(*Penicillium* spp.和 *Fusarium fujikuroi* 等)次级代谢的全局性调控因子[69-73]。LaeA 是在 *aflR* 基因缺失的 *A. nidulans* 突变菌株中发现的一种蛋白，是异质三聚体(Heterotrimeric nuclear complex，the velvet complex)的重要组成成分[70-74]。LaeA 发生组蛋白修饰，增加了染色体重组的激活物，从而激活次级代谢基因簇的表达[75-76]。

LaeA 和 VeA 是影响黄曲霉菌感染寄主作物和黄曲霉毒素污染的重要蛋白因子[77-79]。LaeA 调控 AFT、CPA、曲酸、黄曲霉震颤素等 24 个次级代谢基因簇的表达[79]；VeA 调控 AFT、CPA 和黄曲霉震颤素的生物合成[80]。菌核是黄曲霉菌越冬的形态，对该病害发生周期的循环具有重要作用，并

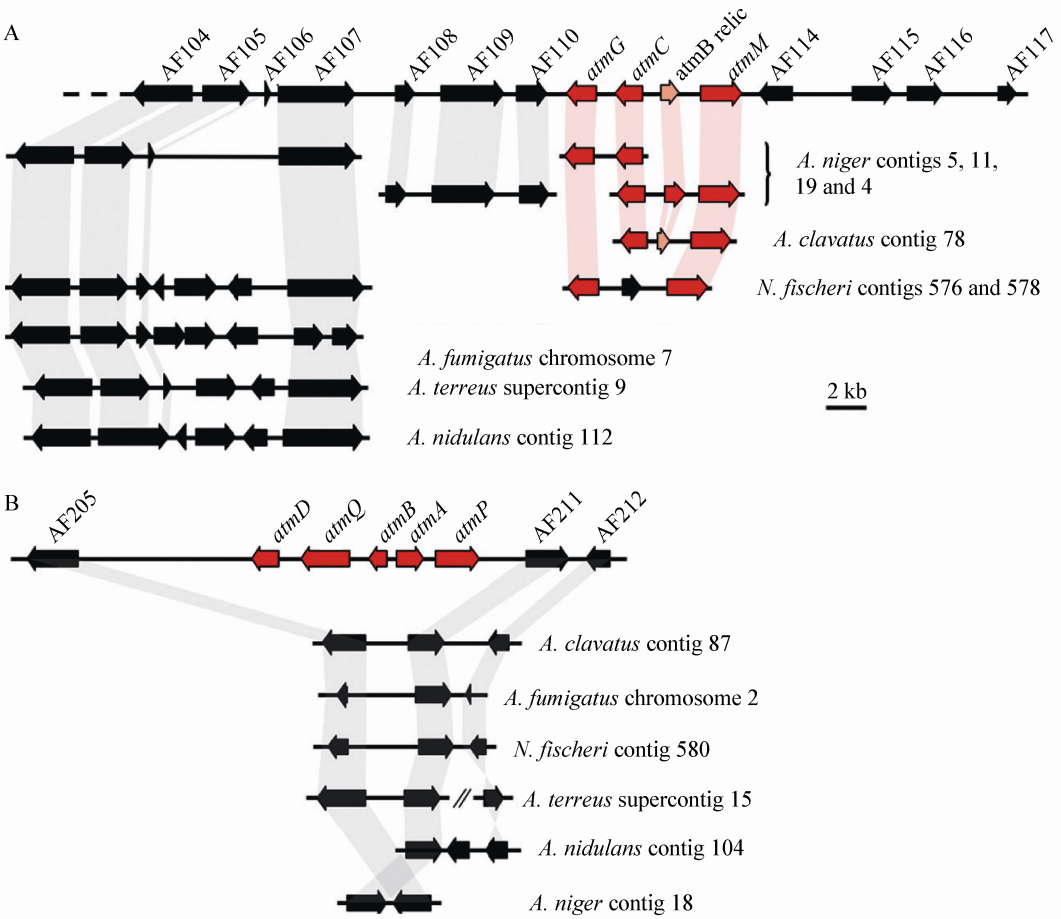

图 5 曲霉菌黄曲霉震颤素次级代谢基因簇及其比较[67]

Figure 5 Physical maps of ATM1 (A) and ATM2 (B) aflatrem biosynthesis loci in *A. flavus* (NRRL6541, NRRL3357) and *A. oryzae* (RIB40) and syntenic regions from other *Aspergillus* genomes[67]

注：红色标识的基因为黄曲霉震颤素合成基因，阴影所标识的部分为不同曲霉菌的同源基因. A 和 B 分别为黄曲霉菌(NRRL6541，NRRL3357)和米曲霉菌(RIB40)为的 ATM1、ATM2 基因簇，其他图例所示为另外几种曲霉菌的黄曲霉震颤素合成基因簇.

Note: The top lines of panels A and B show consensus gene predictions for *A. flavus* NRRL6541, *A. flavus* NRRL3357, and *A. oryzae* RIB40. Proposed aflatrem biosynthesis genes are shown in red, and homology exists over the areas covered by the shaded bands.

且菌核也能分化形成分生孢子和子囊孢子。研究发现，*laeA* 或 *veA* 发生无义突变的黄曲霉菌突变株不仅降低了 AFT、CPA 和黄曲霉震颤素的生成量，并且抑制了菌核的形成；与之相反，*laeA* 或 *veA* 的过量表达增加了黄曲霉毒素合成量和菌核数量[78-81]。

3.2 脂氧合物

脂质是真菌-寄主互作过程中的重要信号分子[82-83]。黄曲霉菌与其寄主作物(花生、玉米等)均含有高水平的亚油酸(C18:2)和油酸(C18:2)等不饱和脂肪酸[84-85]。脂氧合酶催化不饱和脂肪酸产生脂氧合物，脂氧合物(Oxylipins)是丝状真菌、酵母、卵菌、植物和动物信号转导途径的重要信号分子[86]。

Ppo (真菌)和 *lox* (植物、动物和真菌)编码合成的加氧酶类及其氧化产物(脂氧合物，Oxylipins)具有调控黄曲霉菌分生孢子、菌核和次级代谢的作用[87-88]。在构巢曲霉(*A. nidulans*)中首次发现了由 Ppo 蛋白催化产生的脂氧合物(性早熟诱导因子，psi)[89]。黄曲霉菌有 4 种双加氧酶(PpoA、PpoB、PpoC 和 PpoD)和一种脂氧合酶(LoxA)[88,90]，*ppoA* 编码合成 5,8-亚油酸双氧合成酶(PpoA)，*ppoC* 编

码亚油酸-10R-双氧合成酶(PpoC)[91-92]。Horowitz 等研究发现，*ppoA* 和 *ppoC* 无义突变的黄曲霉菌株，分生孢子量减少而菌核量增加；*ppoD* 无义突变菌株的表型变化恰与 *ppoA* 和 *ppoC* 突变菌株相反，菌核量增加，分生孢子量减少[91]。4 个双加氧酶基因(*ppoA*、*ppoB*、*ppoC* 和 *ppoD*)的敲除与 *lox* 基因的突变，均能导致突变菌株黄曲霉毒素含量和菌核量的增加。*ppo* 和 *lox* 突变菌株表型变化进一步证明了脂氧合物对黄曲霉菌发育及次级代谢的影响。

真菌的脂氧合物与动、植物的脂氧合物结构相似，这是脂氧合物作为曲霉-寄主互作信号分子的重要特征[93-95]。植物来源的脂氧合物，如 13S-脂肪酸氢过氧化物(13S-HPODE)和 9S-脂肪酸氢过氧化物(9S-HPODE)，不仅参与植物生长发育和防御反应，而且影响黄曲霉菌、寄生曲霉菌和构巢曲霉的营养生长、分生孢子和菌核的形成及次级代谢反应[87,96]。ZmLOX3 失活的玉米植株更易受 *A. flavus* 侵染，且 AFT 含量增加[94]。然而，构巢曲霉表达植物的脂氧合酶(ZmLOX3)基因可消除 *ppoA* 和 *ppoC* 基因缺失引起的黄曲霉毒素含量和菌核量增加的表型变化[94]。黄曲霉菌侵染榛子、杏仁、花生和玉米等作物时，会诱导黄曲霉菌 *ppo* 基因的表达，而抑制寄主作物 *lox* 的表达[96-98]。

3.3 群体感应

群体感应(密度感应，Quorum sensing)是当菌体的数量达到一定密度时发生的感应现象。在一个特定的环境中，当菌体的数量急剧增加时，由它所分泌的信号分子的浓度也会相应升高，当其达到一个阈值时，菌体之间就会通过发送信号来调整它们的共同行为，对较高的细胞密度做出共同的感应。黄曲霉菌分生孢子/菌核生长的转变是由群体密度决定的，群体密度越高，分生孢子的生成量就越多[88]。黄曲霉菌的群体密度不仅使分生孢子/菌核的形成发生改变，而且对次级代谢也有影响，群体密度升高反而降低黄曲霉毒素的生成量[90]。通过对黄曲霉菌细胞感应的研究发现，高群体密度黄曲霉菌的提取物能够诱导黄曲霉菌形成高密度的群体，反之亦然[90]。

3.4 水解酶

黄曲霉菌等曲霉菌代谢产生 α-淀粉酶、果胶酶、蛋白酶和脂肪酶等大量水解酶类，这些水解酶类在真菌侵染寄主作物的过程中发挥重要作用[99]。淀粉酶是淀粉水解过程中一种重要的水解酶，在淀粉培养基上，*amy1* (α-淀粉酶编码基因)发生无义突变的黄曲霉菌菌株的生长受到抑制，但 *amy1* 发生无义突变的黄曲霉菌菌株在胚受损的玉米种子上能产生黄曲霉毒素，而无胚的玉米胚乳则不能代谢产生毒素[100]。在玉米种子中发现了一种 14 kD 的胰蛋白酶/淀粉酶抑制蛋白，这种蛋白因子抑制黄曲霉菌 α-淀粉酶的活性，并对黄曲霉菌分生孢子的萌发和菌丝的生长具有抑制作用[100-101]。黄曲霉菌 α-淀粉酶的活性影响其对寄主胚乳组织的分解利用，从而影响黄曲霉菌的生长代谢。

果胶是植物细胞壁的重要组成成分，是一类半乳糖醛酸的聚合物，所有植物病原物都能分泌产生果胶酶[99]。黄曲霉菌至少能够分泌多聚半乳糖醛酸酶等 3 种果胶酶，其中由 *pecA* 编码的果胶酶(P2c)与黄曲霉菌的侵染能力相关[102]。*pecA* 超表达的黄曲霉菌对寄主细胞间膜系统的破损能力增强，所以能迅速侵染相邻的细胞，在寄主作物组织内定殖，而 *pecA* 发生无义突变的菌株对寄主的侵染能力降低。此外，强致病性的黄曲霉菌菌株的 P2c 果胶酶、淀粉酶和蛋白酶的活性均较高[103]。虽然脂肪酶的活性影响寄主的发病机制[104]，并且在黄曲霉菌和寄生曲霉菌中已成功克隆一个脂肪酶编码基因 *lipA*[105]，然而尚未见有关 *lipA* 表达活性是否影响黄曲霉菌的致病性及黄曲霉毒素生成水平的研究报道[105]。

黄曲霉菌侵染寄主作物是一个复杂而精准的过程[106]。虽然酶活性的改变可以启动寄主的抗性机制以抑制黄曲霉菌的生长和毒素的生成，但酶活性的改变并不是真菌获取生存所需营养物质的限制性因素[31]。

4 结论与展望

黄曲霉菌在自然界中分布广泛，是人、动物及植物的共同病原菌，黄曲霉菌感染及黄曲霉毒素污染不仅严重制约农业生产的发展，而且是危及食品安全和人类健康的重要因素。黄曲霉菌广泛存在于环境中，黄曲霉菌生境中的其他微生物对黄曲霉菌生长及代谢是否存在影响？其他微生物是促进还是抑制黄曲霉菌侵染寄主和毒素生成？无毒黄曲霉菌能够有效防控黄曲霉毒素污染的发生[34]，但无毒菌株是否是通过改变黄曲霉菌周际的微生物群体来控制毒素污染？在已有研究基础上，继续探讨黄曲霉代谢及防控中的问题，有助于我们对黄曲霉菌全面正确的认识，为防控黄曲霉菌侵染和毒素污染开辟新的途径。

黄曲霉菌基因组大小约 37 Mb，含有 13 000 多个功能基因，55 个次级代谢基因簇，次级代谢基因簇的表达受不同环境条件、次级代谢调控因子、酶活性、复杂的脂氧合物转导信号及群体密度效应的调控。目前，仅明确了 AFT、CPA 和黄曲霉震颤素 3 个次级代谢基因簇及其特征，其他大多数次级代谢基因簇的结构与特性仍不明确，深入研究次级代谢基因簇结构与特性对真菌毒素代谢通径的明确及毒素污染的防控具有重要意义。

基因组测序和全基因组表达数据改变了果蝇、拟南芥和人类传统遗传研究的方法，黄曲霉菌基因组测序的完成同样也改变了这一真菌的研究方法。对黄曲霉菌基因组的研究，有助于对不同生境下黄曲霉菌生理特性、形态差异、进化和代谢多样性的明确；曲霉菌基因组的比对分析，有助于未知功能基因的发掘、复杂行为机理的明确，推动黄曲霉繁殖方式的选择、基因沉默、孢子形成、次级代谢、致病机理及其寄主-曲霉互作等领域的研究。黄曲霉菌基因组测序的完成为曲霉菌次级代谢模式的揭示同曲霉菌分子生物学研究的结合提供了一个很好的切入点。此外，黄曲霉菌基因组信息为真菌功能基因组学研究提供了平台，为有害次级代谢物合成机制的阐释、新抗菌药物的研发、作物抗性遗传改良、黄曲霉菌相关疾病(病害)的预防和治疗、以及黄曲霉毒素污染的防控提出了新战略方向。

群体感应能引起细菌和真菌的孢子形成、次级代谢等代谢过程的转变，因此黄曲霉菌群体感应的分子机制将是未来研究的新方向[107]。同样，在黄曲霉菌侵染作物和动物的过程中，黄曲霉菌对寄主代谢的阻抑与应激反应及寄主对黄曲霉菌代谢的响应等发挥着重要的作用。随着基因敲除、次级代谢基因簇诱导沉默和化学物质检测技术的快速发展，没有功能和特征注释基因簇的研究将取得重大突破。次级代谢基因簇结构和功能的明确，有助于正确认识黄曲霉菌致病性，并具有农业和制药业生产应用的实践价值。

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