特约评述

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米曲霉异源表达天然产物研究进展

董佳钰[1]，李敏[1]，肖宗华[1]，胡明[1]，松田侑大[2]，汪伟光[1]
（[1] 云南民族大学，国家民委民族药内生菌天然产物合成生物学重点实验室，教育部国家民委民族药资源化学重点实验室，云南，昆明 650031；[2] 香港城市大学化学系，香港 999077）

摘要：天然产物是创新药物和生物农药研发的重要源泉。阐明天然产物生物合成关键基因的功能、解析其生物合成通路和酶催化机制对于促进功能天然产物的应用和开发至关重要。异源表达是研究天然产物生物合成和合成生物学的重要手段之一。近年来，米曲霉异源宿主得到了广泛的应用。通过基因工程技术，将目的天然产物生物合成基因和基因簇在米曲霉中异源表达，不仅能够有效地激活沉默的生物合成基因和基因簇，挖掘全新活性天然产物，而且可以快速高效地鉴定天然产物生物合成基因功能，解析和重构其生物合成途径。米曲霉异源表达宿主已经成为天然产物合成生物学研究的强有力工具。本文对米曲霉遗传转化系统在天然产物研究中的应用进行了系统综述。首先，概述了异源表达的应用和意义，介绍了米曲霉遗传转化系统的发展过程、应用基础和优势以及遗传转化方法的实践和优化。其次，根据不同天然产物的结构类型和与之相对应的合成酶特点，着重介绍了该体系表达各类天然产物的成功案例。最后，对米曲霉异源表达宿主在天然产物化学领域的研究和应用前景进行了总结和展望。随着基因编辑、定向进化、合成生物学、生物信息学技术以及人工智能技术的发展和应用，米曲霉异源表达宿主的发展和完善将会极大地促进更多天然产物化学研究技术的发展和革新，以期为天然产物合成生物学的研究和创新药物研发提供借鉴。

关键词：米曲霉；异源表达；天然产物；生物合成；合成生物学

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Recent advances in heterologous production of natural products using *Aspergillus oryzae*

DONG Jiayu[1], LI Min[1], XIAO Zonghua[1], HU Ming[1], Yudai MATSUDA[2], WANG Weiguang[1]
([1]*Key Laboratory of Natural Products Synthetic Biology of Ethnic Medicinal Endophytes, State Ethnic Affairs Commission, and Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission and Ministry of Education, Yunnan Minzu University, Kunming 650031, Yunnan, China*; [2]*Department of Chemistry, City University of Hong Kong, Kowloon, Hong Kong SAR 999077, China*)

Abstract: Organic molecules produced by living organisms, generally termed as natural products, are rich sources of pharmaceutical drugs and biopesticides, and fungi are one of the most prolific producers of medicinally important

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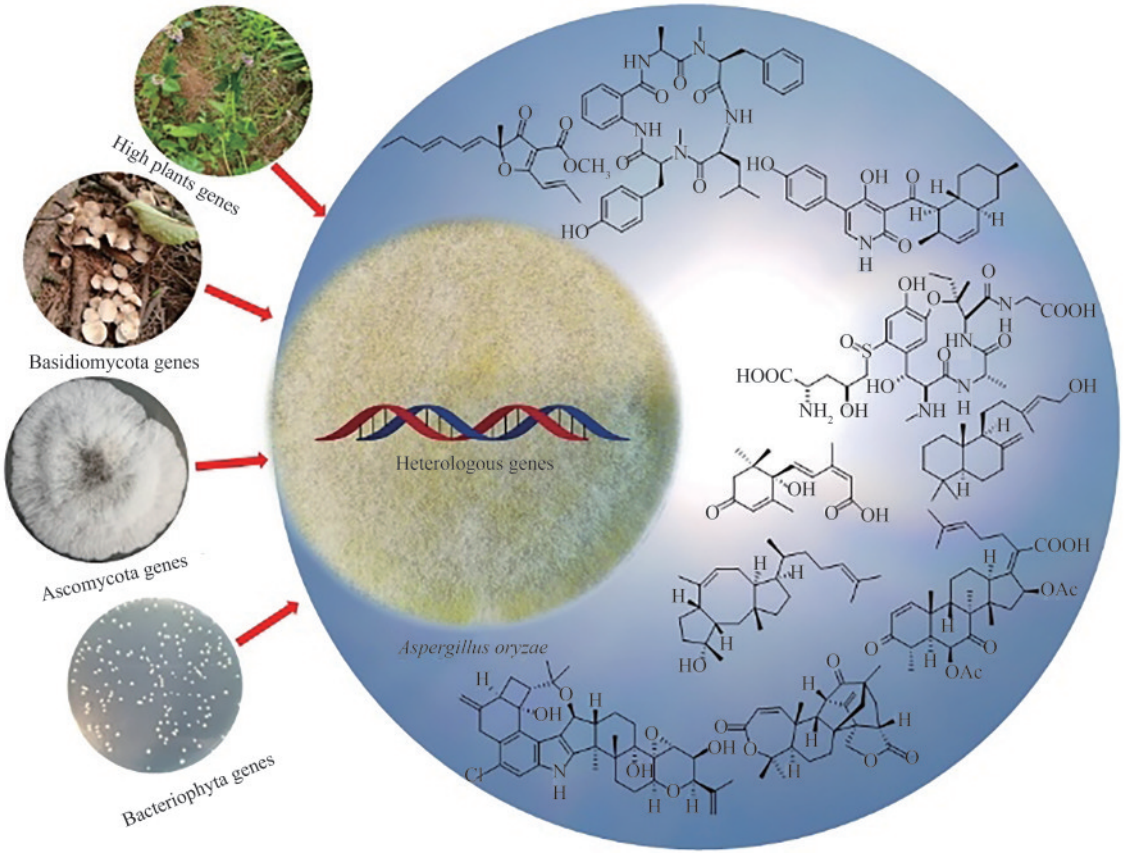
natural products, as represented by penicillins, lovastatin, and cyclosporins. Heterologous expression is a commonly used approach to study the function of biosynthetic genes of natural products, and a number of heterologous hosts have been developed and utilized over the last decades. The filamentous fungus *Aspergillus oryzae* has long been utilized for the production of fermented food and drinks in East Asia, and intensive genetic and molecular biological studies on the fungus have allowed for its genetic engineering in an efficient manner. Importantly, *A. oryzae* is known to possess a relatively clean metabolic background with a low level of secondary metabolite production, providing an attractive feature as a heterologous host. Furthermore, unlike prokaryotic and yeast hosts, most coding sequences of fungal biosynthetic proteins can be directly introduced into *A. oryzae* in their intact form without removing introns, which simplifies the transformation procedures. Collectively, *A. oryzae* is a robust platform for heterologous production of natural products, which not only facilitates the elucidation of the biosynthetic pathway of a given natural product but also allows the activation of silent or cryptic biosynthetic gene clusters. Thus, the *A. oryzae* host has been widely utilized for biosynthetic studies, genome mining, and synthetic biology of fungal natural products. It should be noted that more than ten biosynthetic genes can readily be introduced into the fungus, indicating that the majority of fungal biosynthetic gene clusters can be easily transferred to the *A. oryzae* host. This review first provides the general transformation procedure of *A. oryzae* and the molecular biological tools available for the fungus. Next, recent successful applications of this fungal host for the heterologous production of natural products are summarized. With the recent rapid advance in molecular biology, such as the development of genome editing tools, we believe that the heterologous expression of biosynthetic genes in *A. oryzae* will be performed in a much faster and more versatile manner in the near future, which would ultimately lead to the discovery of useful natural products for drug leads and other applications.



Keywords: *Aspergillus oryzae*; heterologous expression; natural products; biosynthesis; synthetic biology

异源表达是借助于基因工程手段将生物体本体蛋白在异源工程宿主如烟草、大肠杆菌、酵母菌、人源细胞、昆虫细胞等体系中外源高效率表达的手段，可使人们不再过分依赖于原始宿主。该技术已经在生物医药、食品、工业酶等诸多领域得到了广泛的应用，也是合成生物学研究和应用不可或缺的重要手段[1-6]。天然产物一直都是创新药物发现的重要源泉，然而野生宿主中的天然产物含量往往较低，复杂天然产物的化学合成所带来的环境污染、能耗等诸多因素极大地限制了其广泛应用。因而，破解天然产物生物合成基因密码、借助于异源表达策略重构其生物合成途径对于发现结构新颖的活性天然产物、提高其产量、促进创新药物的研发和应用具有非常重要的现实意义[5-8]。

Gomes和Frommer非常系统地比较了现有天然产物异源表达宿主诸如大肠杆菌、酿酒酵母、丝状真菌、植物、哺乳动物细胞等体系的应用范围和优缺点[1-2]，相较于酵母体系，丝状真菌系统因其自带真核基因内含子剪切酶，具有其先天的优势和便利，从而越来越受到人们的关注[1-2, 9]。米曲霉（*Aspergillus oryzae*）是传统发酵和食品加工业的重要菌种，广泛用于食品、饲料、曲酸生产、酿酒等发酵工业，在日本素有“国菌”的美誉[10]，被美国食品药品管理局（Food and Drug Administration，FDA）及世界卫生组织列为食品级安全菌株（generally recognized as safe，GRAS）。米曲霉具有完整的翻译后修饰系统，强大的蛋白生产能力以及分泌能力，尤其是其中能够分泌蛋白酶、淀粉酶、糖化酶和果胶酶，因此被广泛应用于细胞工厂生产多种酶制剂[11-13]。2005年，全基因组测序表明米曲霉的基因组大小为37.9 Mb，包含8条染色体和12 000多个基因[14-15]，是酿酒酵母基因组大小的3倍，与同源性较近的构巢曲霉和烟曲霉相比，米曲霉基因组要大7～9 Mb。由于其遗传背景清晰，自带内含子剪切酶，遗传操作简单，且具有十分完整的初级代谢产物生成能力，故而相较于原核表达体系，米曲霉宿主具有其固有优势。

近年来，用米曲霉作为宿主表达外源蛋白的研究越来越受到重视。米曲霉发酵后几乎很少产生宿主自身次级代谢产物，但是它可以通过引入外源生物合成酶基因产生大量的外源次级代谢产物，如霉菌毒素（如黄曲霉毒素）、药用化合物（如洛伐他汀和大黄素）等活性物质[16]。随着后基因组时代的到来，通过构建和优化异源生物合成途径，从而发现和生产更多新颖结构的生物活性物质，是合成生物学研究的主要目的之一。米曲霉宿主因为其强大的天然产物生物合成酶表达生产能力，在天然产物生物合成和合成生物学领域具有非常重要的研究和应用价值[17-19]。本文首次根据不同天然产物的结构类型和来源，深入系统地综述了米曲霉遗传转化体系在异源表达天然产物领域的应用，着重介绍了聚酮合酶（PKSs）、非核糖体多肽合成酶（NRPSs）、聚酮合酶-非核糖体肽合成酶杂合（PKS-NRPSs）、倍半萜烯合成酶、二萜烯合成酶、二倍半萜合成酶、三萜合成酶和杂萜合成酶等生物合成基因和基因簇在米曲霉中异源表达的研究进展。

1 米曲霉遗传转化系统

米曲霉遗传转化宿主多由*A. oryzae* RIB40菌株基础上开发而来，现有*A. oryzae* NID300、*A. oryzae* MS4、*A. oryzae* NSR13、*A. oryzae* NSA1、*A. oryzae* NSA2、*A. oryzae* NSAR2等菌株序列[16, 20]。目前在天然产物生物异源表达领域应用最为广泛的米曲霉宿主为*Aspergillus oryzae* NSAR1（*niaD*$^{-}$，*sC*$^{-}$，Δ*argB*，*adeA*$^{-}$）系统，该系统最早为东京大学Kitamoto教授和东北大学Gomi教授开发完善而来[20-22]。该宿主同时具有硝酸盐还原酶营养缺陷型（*niaD*$^{-}$）[23]、甲硫氨酸营养缺陷型（*sC*$^{-}$）[24]、精氨酸营养缺陷型（Δ*argB*）[21]以及腺嘌呤营养缺陷型（*adeA*$^{-}$）[22]四种缺陷基因筛选标记。随后Kubodera等和Matsuda等相继将吡啶硫胺抗性基因*ptrA*[25]和草铵膦抗性基因*bar*[26]应用于米曲霉宿主，使得该体系进一步完善。2014年，Matsuda等[26]成功运用如上六种筛选标记依托*A. oryzae* NSAR1完成了复杂天然产物anditomin的生物合成研究，同时异源表达外源基因11条，再次证明了该体系在异源表达复杂天然产物研究领域的潜在价值，为进一步深入和优化研究该体系奠定了有力的基础。

2 生物合成基因簇在米曲霉中异源表达的研究进展

由于丝状真菌具有细胞壁，很难实现类似于大肠杆菌的热激转化，常需要借助于外加压力的方式增加转化效率，目前米曲霉的异源表达天然产物生物合成基因主要采用PEG/$CaCl_2$介导的原生质体转化法[27]。运用分子生物学手段，首先将外源基因装载在如上所述的几种营养缺陷型筛选标记和抗性标记基因载体上，借助于转化试剂的高糖渗透压将目标基因载体转化到原生质体细胞中。携带目标基因的载体与宿主基因组发生同源重组后就可以将目标基因插入到米曲霉的染色体中。目前常用于*A. oryzae* NSAR1的表达载体主要有6种，分别为pTAex3（Δ*argB*）、pUSA（*sC*$^-$）、pUNA（*niaD*$^-$）、pAdeA（*adeA*$^-$）、pPTRI（*ptrA*）和pBARI（*bar*）[26]。除上述常用表达载体外，Oikawa课题组还在米曲霉中成功使用过表达载体pUARA2（*ΔargB*）[28]、pTASU03（Δ*argB*）[29]、pDP801（*sC*$^-$）[30]等。Cox课题组[31]也在米曲霉中成功表达过pTYGS系列等载体。转化基因和基因簇既可以单个插入也可以一整条插入，例如Nihira等[32]利用cosmid建库的方式分别将*Monascus pilosus*中负责生物合成monacolin K和*Aspergillus nidulans*中负责生物合成terrequinone A的一整条基因簇（40 kb和12 kb）装载到载体中后，成功实现了两条基因簇在米曲霉宿主中的异源表达。单个基因插入则是将目的基因5′端和3′端分别添加淀粉酶（AmyB）[26]或改进型淀粉酶[33]增强型启动子和终止子，再利用培养基中的淀粉或者糊精加以诱导，就能轻松实现目的基因的高效率表达[29, 34]。

原生质体转化的最大优点在于成熟的转化体系下，目标基因的插入便于筛选得到高表达基因菌株，而其缺点在于遗传稳定性因素影响，隔代遗传有可能会导致目标基因的丢失或不表达。故而针对该系统进行优化的研究非常有必要，例如近期Oikawa等和Matsuda等相继利用改进型CRISPR-Cas9基因编辑技术成功在*A. oryzae*宿主中实现了基因敲入[30, 35]。该方法的优点在于可以轻松实现外源基因在米曲霉基因组中的定点插入，插入效率高，节省大量筛选时间，且在外源基因插入后可以自发去除筛选标记，使得插入基因不受筛选标记有限的影响，便于多基因的相继插入，尤其适用于多基因天然产物生物合成基因簇的异源表达[30]。

2.1 聚酮化合物

聚酮类（polyketides）化合物是非常常见的天然产物，种类繁多，目前已发现的聚酮化合物超过10 000个[36-37]。聚酮化合物具有广泛的用途，特别是在医药领域，每年聚酮类药物例如洛伐他汀、红霉素、阿奇霉素等的销售额超过200亿美元[38]。随着合成生物学的发展及绿色制造的兴起，越来越多的研究人员通过对聚酮类化合物的合成方法不断改造和优化，为开发更多的聚酮化合物的合成方法提供了新思路。本部分系统整理了近年来利用米曲霉系统异源表达得到的代表性聚酮类化合物的结构、基因来源以及基因和基因簇特点（图1和表1）。

Watanabe等[39]在米曲霉中异源表达了烟曲霉PKS途径的*alb1*基因，生成化合物YWA1（**1**）及其对映异构体。Cox等[40]首次在米曲霉中异源表达*Phoma* sp. C2932中包含KS、AT、DH、*C*-MeT、ER、KR和ACP结构域编码区域完整的聚酮合成酶PKS基因簇，产生squalestatin S1的生物合成中间体化合物**2**。Seshime等[41]在米曲霉异源表达过程中，发现了III型聚酮合酶CsyA参与3，5-dihydroxybenzoic acid（**3**）的生物合成。Awakawa等[42]从*Nectria haematococc*a mpVI 77-13-4中发现一个具有聚酮合成酶（PKS）与巯基还原酶（TR）功能的基因（NhPKS1），并在米曲霉中进行异源表达，得到了一个中间体化合物**4**。Wattanachaisaereekul等[43-44]通过米曲霉实现了anhydromevalonolactone（**5**）的生物合成。Ugai[45]以米曲霉为异源表达宿主，证实了*bet*基因簇中高度还原聚酮合成酶基因（*bet1*）和反式烯酰还原酶基因（*bet3*）参与了betaenone B（**6**）的生物合成。Hashimoto等[46]对*Talaromyces stipitatus* ATCC 10500中C末端甲基转移酶结构域的NR-PKS基因进行功能分析，并在米曲霉中表达了*tspks3*基因，进而生物合成了2-acetyl-7-methyl-3，6，8-

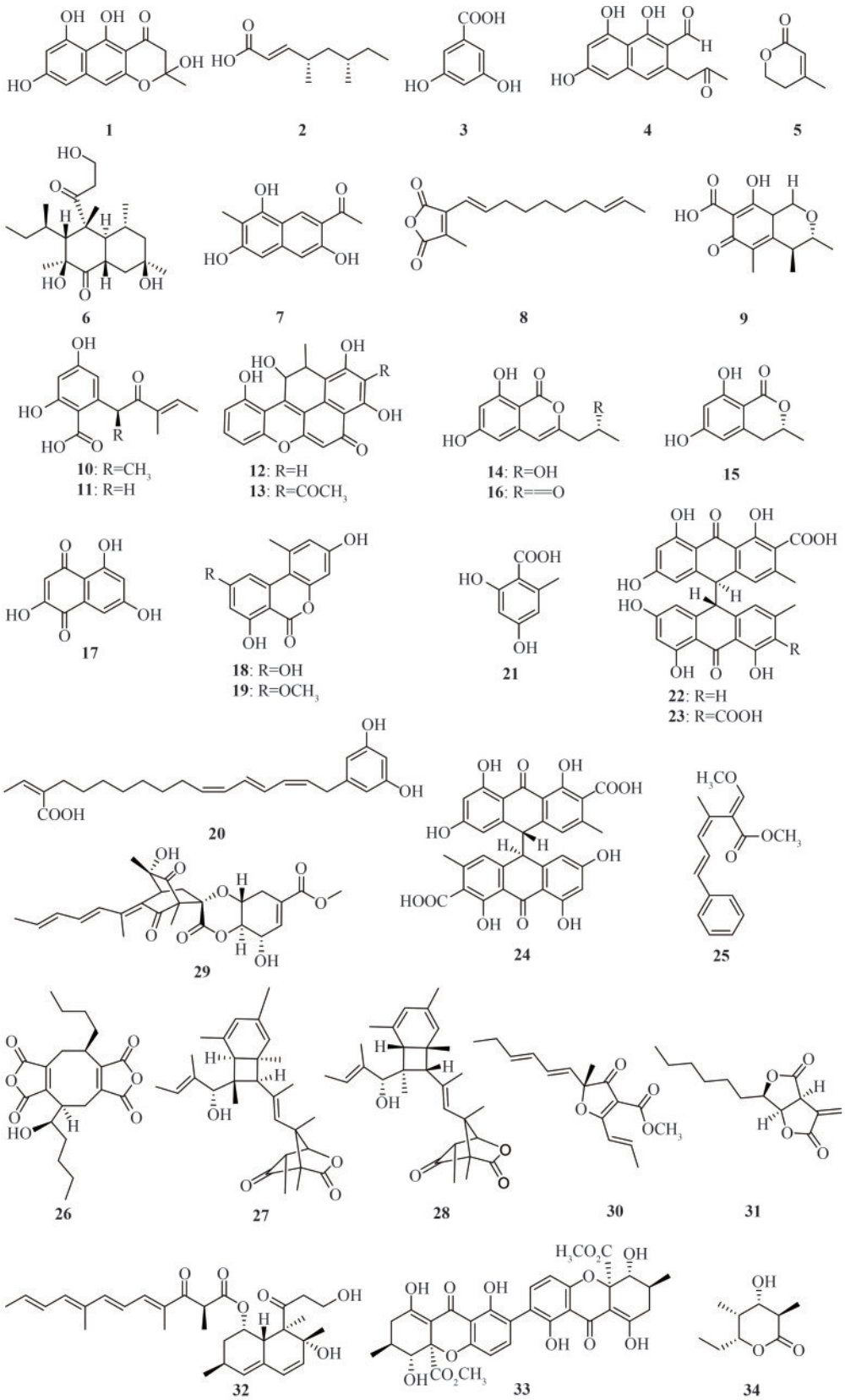


图1 经由米曲霉系统异源表达的代表性聚酮类化合物结构

Fig. 1 Structures of polyketides heterologously produced in the *Aspergillus oryzae* system

表1 米曲霉异源表达的代表性聚酮类化合物来源基因以及宿主信息

Tab. 1 Representative polyketides heterologously produced in *Aspergillus oryzae*, origins of the biosynthetic genes, and hosts used for the heterologous expression

化合物名称和编号	基因来源	表达宿主	参考文献
YWA1 (**1**)	*Aspergillus fumigatus*	*A. oryzae* M-2-3	[39]
2	*Phoma* sp. C2932	*A. oryzae* M-2-3	[40]
3,5-dihydroxybenzoic acid (**3**)	*Aspergillus oryzae* RIB40	*A. oryzae* M-2-3	[41]
4	*Nectria haematococca* mpVI 77-13-4	*A. oryzae* NSAR1	[42]
anhydromevalonolactone (**5**)	*Xylaria* sp. BCC 1067	*A. oryzae* U1638	[43-44]
betaenone B (**6**)	*Phoma betae Fr.*	*A. oryzae* NSAR1	[45]
2-acetyl-7-methyl-3,6,8-trihydroxynaphthalene (**7**)	*Talaromyces stipitatus* ATCC 10500	*A. oryzae* M-2-3	[46]
8	unidentified fungus ATCC 74256	*A. oryzae* NSAR1	[47]
citrinin (**9**)	*Monascus ruber* M7	*A. oryzae* NSAR1	[48]
10和**11**	*Arthrinium* sp. NF2194	*A. oryzae* NSAR1	[49]
dalmanol A (**12**) acetodalmanol A (**13**)	*Daldinia eschscholzii*	*A. oryzae* NSAR1	[50]
(−)-orthosporin (**14**) (−)-6-hydroxymellein (**15**)	*Menisporopsis theobromae* BCC 4162	*A. oryzae* NSAR1	[51]
benzopyran (**16**) flaviolin (**17**)	*Cladosporium fulvum*	*A. oryzae* M-2-3	[52]
alternariol (**18**) alternariol monomethyl ether (**19**)	*Alternaria alternata* ATCC 66981	*A. oryzae* NSAR1	[53]
soppiline C (**20**)	*Penicillium soppi* Okera-1	*A. oryzae* NSAR1	[54]
orsellinic acid (**21**)	*Fusarium* sp. NBRC100844	*A. oryzae* NSAR1	[55]
22-24	*Aspergillus terreus*	*A. oryzae* Δ*snfA*Δ*SCAP*	[56]
strobilurin A (**25**)	*Strobilurus tenacellus*	*A. oryzae* NSAR1	[31]
zopfiellin (**26**)	*Zopfiella curvata* No. 37-3	*A. oryzae* NSAR1	[57]
shimalactone A (**27**)、shimalactone B (**28**)	*Emericella variecolor* GF10	*A. oryzae* M-2-3	[58]
spirosorbicillinol B (**29**)	*Trichoderma reesei* QM6a	*A. oryzae* NSAR1	[59]
gregatin A (**30**)	*Penicillium* sp. sh18	*A. oryzae* NSAR1	[60]
sporothriolide (**31**)	*Hypomontagnella monticulosa* MUCL 54604	*A. oryzae* NSAR1	[61]
32	*Emericella variecolor* NBRC 32302	*A. oryzae* NSAR1	[62]
secalonic acid D (**33**)	*Aspergillus aculeatus* CBS 172.66	*A. oryzae* NSAR1	[33]
TKL (**34**)	*Saccharopolyspora erythraea*	*A. oryzae* NSAR1	[63]

trihydroxynaphthalene （**7**）。Fujii等[47]在米曲霉中异源表达了来源于未鉴定的真菌ATCC 74256的*phi*基因簇，该基因簇编码高度还原聚酮合成酶（PhiA）、甲基柠檬酸合酶（PhiJ）和甲基柠檬酸脱氢酶（PhiI），从而产生了化合物**8**。He等[48]在米曲霉中异源表达出*Monascus ruber* M7的以非还原聚酮合酶基因（*pksCT*）为核心的基因簇，产生了PKS的化合物citrinin （**9**）。Zhang等[49]将*Arthrinium* sp. NF2194的聚酮合成酶基因（*atnGH*）在米曲霉中异源表达，获得了两个聚酮类中间产物**10**和**11**。Zhou等[50]在米曲霉异源表达实现了dalmanol A （**12**）和acetodalmanol A （**13**）的生物合成。Bunnak等[51]在米曲霉中同时表达了*Menisporopsis theobromae* BCC 4162中的高度还原聚酮合酶基因（*men1*）和非还原聚酮合酶基因（*men2*），得到了化合物(−)-orthosporin （**14**）和(−)-6-hydroxymellein （**15**）。Griffiths等[52]在米曲霉中实现了异源表达番茄真菌病原体*Cladosporium fulvum*的*CfPKS1*基因簇，完成化合物benzopyran （**16**）和flaviolin （**17**）的生物合

成。Wenderoth等[53]在米曲霉实现了植物感染过程中发挥作用的化合物alternariol（**18**）和alternariol monomethyl ether（**19**）的生物合成。Kaneko等[54]在米曲霉中异源表达了来源于青霉菌*Penicillium soppi* Okera-1的*psp*基因簇，从而产生了soppiline C（**20**）。orsellinic acid（**21**）是利用*Fusarium* sp. NBRC100844中编码聚酮合酶的基因（*OAS*）在米曲霉中异源表达得到的[55]。Kan等[56]在米曲霉异源表达过程中，发现了丙二酰辅酶A高效参与蒽酮二聚体（**22**～**24**）的生物合成。Lebe等[31]在米曲霉中异源表达了含有高度还原聚酮合成酶基因（*stpks1*）的基因簇，产生具有很强抑菌活性的化合物strobilurin A（**25**）。Shiina等[57]通过米曲霉实现了多功能双加氧酶的氧化环收缩功能，完成了化合物zopfiellin（**26**）的生物合成。Fujii等[58]通过米曲霉表达*shmA*和*shmB*基因，重构了shimalactone A、shimalactone B（**27**和**28**）的生物合成。Kahlert等[59]根据*Penicillium chrysogenum*中合成sorbicillinoid的基因簇，使用在*Trichoderma reesei* QM6a找到的同源基因簇，在米曲霉中进行异源表达，从而产生了spirosorbicillinol B（**29**）。汪伟光课题组及合作伙伴[60]以米曲霉为异源表达宿主，证实了gregatin A（**30**）的生物合成途径是由聚酮合成酶（GrgA）、反式烯酰还原酶（GrgB）、α/β水解酶（GrgF）调控生成。Tian等[61]通过米曲霉异源表达*spo*基因组合，实现了化合物sporothriolide（**31**）的生物合成。Tao等[62]为探索化合物calbistrin骨架结构生成途径，在米曲霉中异源表达完成类似物**32**的生物合成，发现单个PKS酶可以合成两种不同结构的产物。Wei等[33]在米曲霉异源表达实现secalonic acid D（**33**）的生物合成。Feng等[63]用米曲霉将细菌模块化聚酮合酶DEBS1-TE进行了异源表达，改变了真菌米曲霉的代谢，得到三酮内酯化合物triketide lactone（TKL；**34**），表明米曲霉可以用作细菌来源聚酮化合物的异源表达宿主。

2.2 非核糖体多肽

非核糖体多肽合成酶（non-ribosomal peptide synthetases，NRPSs）在细菌和真菌中广泛存在，以不同的氨基酸为底物催化产生缩合肽类天然产物。肽类化合物及衍生物按其合成途径可以分为两类：一类是通过核糖体对多肽进行缩合修饰及环化形成；另一类是通过模块化的非核糖体多肽合酶复合体（NRPS）将蛋白或非蛋白氨基酸逐一组装起来。NRPS主要由不同的独立模块组成，每个模块中都含有选择和活化特殊的氨基酸的腺苷酰化结构功能域（adenylation，A），将氨酰残基加载至巯基化的结构功能域（thiolation，T）上，以及将活化的氨基酸进行聚合从而产生酰胺类的肽类化合物的缩合结构功能域（condensation，C）[64-65]。de Mattos-Shipley等[66]利用米曲霉成功表达了*Penicillium soppii* CBS 869.70的*Pscy*基因簇，该基因簇由非核糖体肽合酶和新型反式*N*-甲基转移酶（*N*-MeT）组成，从而生物合成了cycloaspeptide A（**35**）和cycloaspeptide E（**36**）。Yoshimi等[67]将其在真菌*Curvularia clavata*中预测的基因簇导入米曲霉中，得到了异源表达化合物KK-1（**37**）（图2和表2）。

2.3 核糖体多肽

核糖体肽类化合物（RiPPs）是一类特殊编码的多肽经由一系列后修饰酶加工而成，常见于细菌次级代谢产物，具有广泛的生物活性，目前真菌中只有为数不多的例子报道[68]，该类化合物在米曲霉中的异源表达工作均系Oikawa教授课题组完成。该组首先在米曲霉中异源表达*Aspergillus flavus* CA14的*ust*基因簇，发现了大环化酶、5-磷酸吡哆醛酶（UstD）和3种氧化酶（UstC、UstF1、UstF2）参与ustiloxin B（**38**）的生物合成[69]。而后，又通过米曲霉异源表达，发现DUF3328蛋白AprY参与了特征双环结构的形成，得到化合物asperipin-2a（**39**）[70]（图2和表2）。

2.4 聚酮和非核糖体多肽杂合化合物

真菌聚酮合酶-非核糖体肽合成酶（PKS-NRPSs）是一种多结构域的大型合成酶，PKS-NRPS杂合酶由PKS单元（KS，AT，DH，*C*-MeT，KR和ACP等结构域）和NRPS单元（A，T和C等结构域）组成。PKS-NRPS具有催化PKS产物与NRPS产物结合的功能，从而产生结构复杂多样且具有广泛生物活性的天然产物，是活性先导化合物的重要源泉[71-72]。Seshime等[73]将*Aspergillus*

flavus NRRL 3357的*cpaA*基因在米曲霉中进行异源表达，得到了cyclopiazonic acid生物合成中间体cAATrp（**40**）作为PKS-NRPS的特异性产物。Heneghan等[74]在米曲霉中异源表达出*Beauveria bassiana* 110.25的*ten*基因簇，生成了3个具有抗真菌的化合物pretenellin A（**41**）、pretenellin B（**42**）和tenellin（**43**）。Fisch等[75]在米曲霉中异源表达出*Beauveria bassiana* 992.05的*tenS*基因，从而实现了pretenellin A（**41**）和desmethyl-pretenellin A（**44**）的生物合成。Fujii等[76]将*Aspergillus clavatus* NRRL1的基因*ccsA*和*ccsC*导入米曲霉进行异源表达，得到了化合物**45**。Wasil等[77]在米曲霉中导入来自*Aspergillus nidulans* SB4.1的*apd*基因簇，得到了包括aspyridone A（**46**）在内的9个化合物。Song等[78]在米曲霉中共表达*Magnaporthe oryzae*中的去内含子PKS-NRPS *ACE1*基因簇和反式还原酶（*trans*-ER；*RAP1*），产生了1个具有细胞松弛素骨干结构的酰胺化合物**47**。Ugai等[79]在米曲霉中异源表达*Alternaria solani* A-17中*asol*基因簇，得到具有抗真菌活性的化合物didymellamide B（**48**）。Zhang等[80]在米曲霉中异源表达出*Tolypocladium* sp. 49Y中PKS-NRPS杂合途径的4-羟基吡啶酮基因簇，生成了4-hydroxy pyridines型化合物tolypyridone C（**49**）、tolypyridone D（**50**）（图2和表2）。

2.5 萜类化合物

萜类化合物是自然界中存在数量仅次于糖类

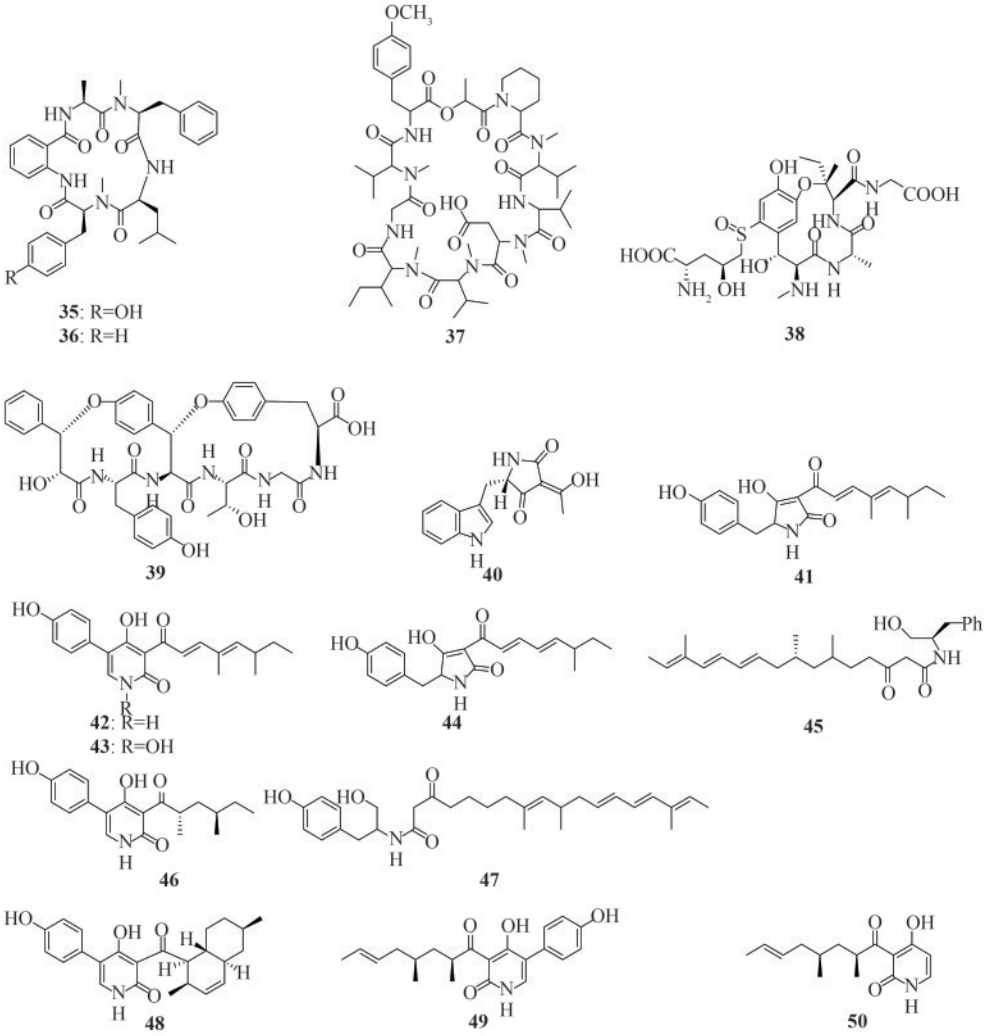


图2 经由米曲霉系统异源表达的代表性肽类化合物结构

Fig. 2 Structures of peptide natural products heterologously produced in the *Aspergillus oryzae* system

表2 米曲霉异源表达的代表性肽类化合物来源基因以及宿主信息

Tab. 2 Representative peptide natural products heterologously produced in *Aspergillus oryzae*, origins of the biosynthetic genes, and hosts used for the heterologous expression

化合物分类	化合物名称和编号	基因来源	表达宿主	参考文献
非核糖体多肽	cycloaspeptide A (**35**) cycloaspeptide E (**36**)	*Penicillium soppii* CBS 869.70	*A. oryzae* NSAR1	[66]
	KK-1 (**37**)	*Curvularia clavata*	*A. oryzae* CNT	[67]
核糖体多肽	ustiloxin B (**38**)	*Aspergillus flavus* CA14	*A. oryzae* NSAR1	[69]
	asperipin-2a (**39**)	*Aspergillus flavus* CA14	*A. oryzae* NSAR1	[70]
聚酮和非核糖体多肽杂合化合物	cAATrp (**40**)	*Aspergillus flavus* NRRL 3357	*A. oryzae* M-2-3	[73]
	pretenellin A (**41**)、pretenellin B (**42**) tenellin (**43**)	*Beauveria bassiana* 110.25	*A. oryzae* M-2-3	[74]
	pretenellin A (**41**) desmethyl-pretenellin A (**44**)	*Beauveria bassiana* 992.05	*A. oryzae* M-2-3	[75]
	45	*Aspergillus clavatus* NRRL1	*A. oryzae* NSAR1	[76]
	aspyridone A (**46**)	*Aspergillus nidulans* SB4.1	*A. oryzae* M-2-3	[77]
	47	*Magnaporthe oryzae*	*A. oryzae* M-2-3	[78]
	didymellamide B (**48**)	*Alternaria solani* A-17	*A. oryzae* NSAR1	[79]
	tolypyridone C (**49**)、tolypyridone D (**50**)	*Tolypocladium* sp. 49Y	*A. oryzae* NSAR1	[80]

化合物的一类具有重要生理功能的天然产物，系由异戊二烯基本单元聚合而成，根据异戊二烯单元聚合数量的差异分为半萜、单萜、倍半萜、二萜、二倍半萜、三萜和甾体等[81-84]，例如薄荷醇（单萜）、著名的抗疟疾药物青蒿素（倍半萜）、维生素A、抗感染类药物穿心莲内酯和抗癌药紫杉醇（二萜）、人参皂苷和诸多的甾体抗炎药等（三萜和甾体类）。由于具有重要的生理功能，萜类化合物一直备受广大研究者关注。萜类环化酶是导致萜类化合物结构多样性的最核心因素，根据其结构域的差异可以分为Ⅰ型、Ⅱ型以及双功能型[85]。由于米曲霉体系拥有几乎所有萜类化合物合成的前体化合物，且无论哪种萜类环化酶均可以在此宿主中得以高效率表达，因此是非常优异的萜类化合物异源表达底盘工具，被广泛应用于研究萜类化合物的生物合成、基因组挖掘以及合成生物学等相关研究。本文根据萜类化合物的分类系统综述了近年来借助于米曲霉宿主，表达该类化合物的研究概况。

2.5.1 倍半萜类

倍半萜类化合物（sesquiterpenoids）是指含15个碳原子的天然萜类化合物，主要用于香料、驱蚊剂、食用色素和高价值治疗分子的工业价值产品[86-91]。Takino等[92]从*Botrytis cinerea* SAS56中发现了倍半萜烯合成酶基因簇，并以米曲霉为异源表达宿主，实现了abscisic acid（**51**）生物合成。Murai等[93]以米曲霉为异源表达宿主，生物合成1个新颖骨架的倍半萜醇trichobrasilenol（**52**）。Lee等[94]以米曲霉为异源表达宿主，发现了萜烯环化酶基因（*aneC*）和三种细胞色素P450酶基因（*aneD*、*aneE*、*aneF*）参与aculene D（**53**）和asperaculane C、asperaculane D、asperaculane E、asperaculane F、asperaculane G（**54**～**58**）的生物合成。Feng等[95]将*Annulohypoxylon truncatum* CBS 140778的萜烯环化酶、P450氧化酶和乙酰氨基葡萄糖酶在米曲霉中异源表达，获得了化合物brasilane A（**59**）、brasilane D（**60**）和brasilane E（**61**）（图3和表3）。

2.5.2 二萜

二萜化合物的分子骨架由4个异戊二烯单元组成（GGPP），具有抗肿瘤、抗炎、免疫抑制剂等多种生物活性[96]。Fujii等[97]将*Phoma betae* PS-13中以二萜合成酶*PbACS*基因为主的基因簇导入米曲霉进行异源表达，生成了aphidicolin（**62**）。Qin等[98]用基因组挖掘的方法，在真菌*Emericella variecolor* NBRC 32302基因组中发现了一个萜烯合成酶基因（*EvVS*），通过米曲霉异源表达，得到了一个新型三环二萜烃variediene（**63**）。Bailey

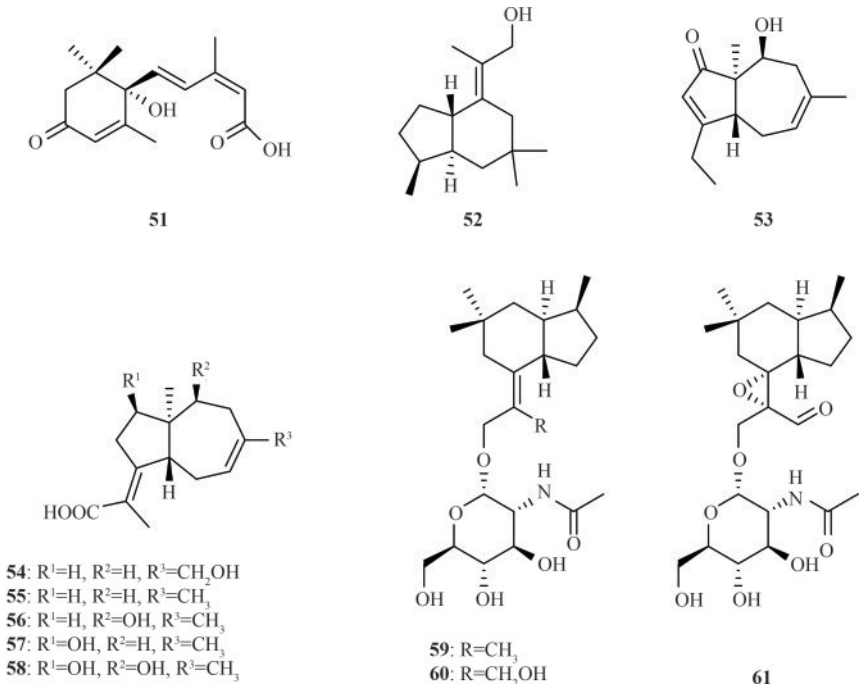


图3 经由米曲霉系统异源表达的代表性倍半萜类化合物结构

Fig. 3 Structures of sesquiterpenoids heterologously produced in the *Aspergillus oryzae* system

等[34]、Yamane等[29]和Alberti等[99]分别以米曲霉为异源宿主，实现了具有生物活性的化合物pleuromutilin（**64**）的生物合成。Mitsuhashi等[100]分别将*Penicillium verruculosum* TPU1311中*PvCPS*基因和*Penicillium fellutanum* ATCC 48694中*PfCPS*基因导入米曲霉进行异源表达，得到了一个相同的二萜醇类化合物(+)-copalol （**65**），证明了*PvCPS*基因和*PfCPS*基因同时具有II型萜烯环化酶和异戊二烯基转移酶功能。Mitsuhashi等[101]用基因组挖掘的方法，在*Penicillium chrysogenum* MT-12中找到了具有萜烯环化酶和异戊二烯基转移酶功能的*PcCS*基因，并在米曲霉中实现了化合物**66**的生物合成。Tazawa等[102]以米曲霉为异源宿主，表达了来源于*Pseudocercospora fijiensis* 10CR-1-24的*bsc*基因簇，实现了brassicicene I （**67**）的生物合成。Liu等[30]在米曲霉中对真菌*Hericium erinaceus* yamabushitake Y2中的*eri*基因簇进行异源表达，得到了化合物erinacine Q （**68**）。Lin等[103]将*Myrothecium graminearum* ZLW0801-19中二萜合酶导入米曲霉进行异源表达，生成myrothec-15(17)-en-7-ol（**69**）（图4和表3）。

2.5.3 二倍半萜

二倍半萜类化合物是一类萜类天然产物，主要以香叶酰基焦磷酸盐（GFPP）为底物进行环化合成[104-105]。Oikawa课题组[106]从*Aspergillus clavatus* NRRL1找到同时具有I类二倍半萜合成酶和异戊烯转移酶功能的*AcOS*基因，并以米曲霉为异源表达宿主，得到了化合物ophiobolin F （**70**），首次利用米曲霉宿主开展了该类化合物的基因组挖掘工作。Quan等[107]在*Aspergillus calidoustus* CBS121601中找到了*AcOS*的同源基因*AcldOS*并在米曲霉中异源表达，从而实现了ophiobolin F（**70**）的生物合成。Matsuda等[108]使用基因组挖掘的方法在*Emericella variecolor* NBRC 32302中找到了*AcOS*同源基因*Stl-SS*，在米曲霉中异源表达得到一个新的三环二萜化合物stellata-2,6,19-triene（**71**）。Matsuda等[109]将*Emericella variecolor* NBRC 32302的萜烯环化酶*EvAS*在米曲霉中异源表达，获得了astellifadiene （**72**）。Okada等[110]从*Emericella variecolor* NBRC 32302中找到二倍半萜合成酶基因簇*EvQS*，并将其导入米曲霉进行异源表达，从而实现了quiannulatene（**73**）和quiannulatic acid（**74**）的生物合成。Narita等[111]将*Bipolaris maydis* ATCC48331中的萜烯合酶BmTS1-3导入米曲霉中异源表达，得到了化合物Bm1（**75**）和Bm3（**76**），将*Phoma betae* PS-13中的*PbTS1*基因

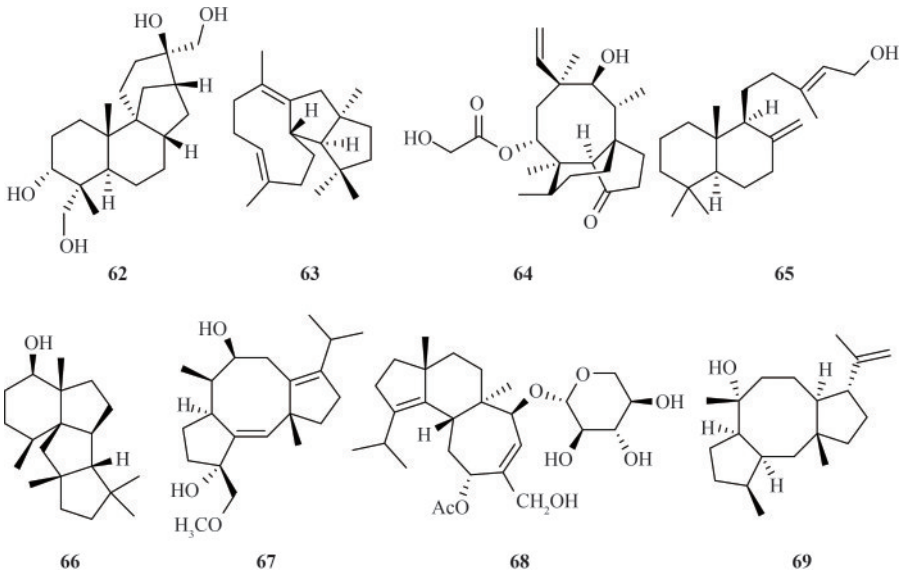


图4 经由米曲霉系统异源表达的代表性二萜类化合物结构

Fig. 4 Structures of diterpenoids heterologously produced in the *Aspergillus oryzae* system

导入米曲霉中，得到化合物Pb1（**77**）。Narita等[112]在*Bipolaris maydis* C5中发现与二倍半萜化合物合成相关的*tpc*基因簇，通过米曲霉异源表达，完成了化合物(−)-terpestacin（**78**）的生物合成。Gao等[113]在Narita的研究基础上，在米曲霉中导入*Phoma betae* PS-13中*btc*基因簇中的萜烯合酶基因（*btcAPb*）和氧化酶基因（*btcBPb*），得到化合物betaestacin Ⅱ（**79**）。Huang等[114]在*Talaromyces wortmannii* ATCC 26942中找到含萜烯合酶基因在内的*ast*基因簇，通过米曲霉异源表达，证明了该基因簇负责化合物asperterpenoid A（**80**）的生物合成。Quan等[115]在米曲霉中异源表达出*Aspergillus calidoustus* CBS121601的一条小基因簇，该基因簇由萜烯环化酶基因（*acldAS*）和P450氧化酶基因（*acldA-P450*）组成，从而实现了asperterpenol A（**81**）、asperterpenol B（**82**）的生物合成。Guo等[116]通过基因组挖掘的方法在*Aspergillus ustus* 094102中找到萜烯合酶基因*AuAS*，该基因在米曲霉中异源表达可以生成化合物aspergiltriene A（**83**）和aspergildiene A（**84**）、aspergildiene B（**85**）、aspergildiene C（**86**）、aspergildiene D（**87**）。Jiang等[117]在*Fusarium oxysporum* FO14005中找到萜烯合酶基因*FoFS*，通过米曲霉异源表达，得到了化合物fusoxypene A（**88**）、fusoxypene B（**89**）、fusoxypene C（**90**）和(−)-astellatene（**91**），在*Aspergillus terreus* NIH 2624中找到萜烯合酶基因*AtAS*，并在米曲霉异源表达中得到化合物preaspterpenacid Ⅰ（**92**）（图5和表3）。

2.5.4 三萜和甾体类

三萜类化合物在自然界中分布广泛，具有多种药理活性，也是天然药物的重要来源[118]。Lv等[119]将*Aspergillus fumigatus* Af293的*hel*基因簇在米曲霉中异源表达，实现了抗生素helvolic acid（**93**）的生物合成。Wang等[120]发现*Nodulisporium* sp.（no. 65-17-2-1）中有细胞色素P450单加氧酶生物合成基因等，将其导入米曲霉异源表达获得了包括化合物**94**在内的14个demethoxyviridin生物合成中间产物。Cao等[121]利用米曲霉成功表达了*Acremonium fusidioides* ATCC 14700的*fus*基因簇，产生了1个具有抑菌活性的三萜类化合物fusidic acid（**95**）。Wang等[122]利用米曲霉成功表达了*Thanatephorus cucumeris* NBRC 6298中*TcP450-1*基因，获得了C19-氧化产物**96**。Cao等[123]将*Acremonium chrysogenum* ATCC 11550的*cep*基因簇在米曲霉中异源表达，获得了fusidane型抗生素cephalosporin P_1（**97**）（图6和表3）。

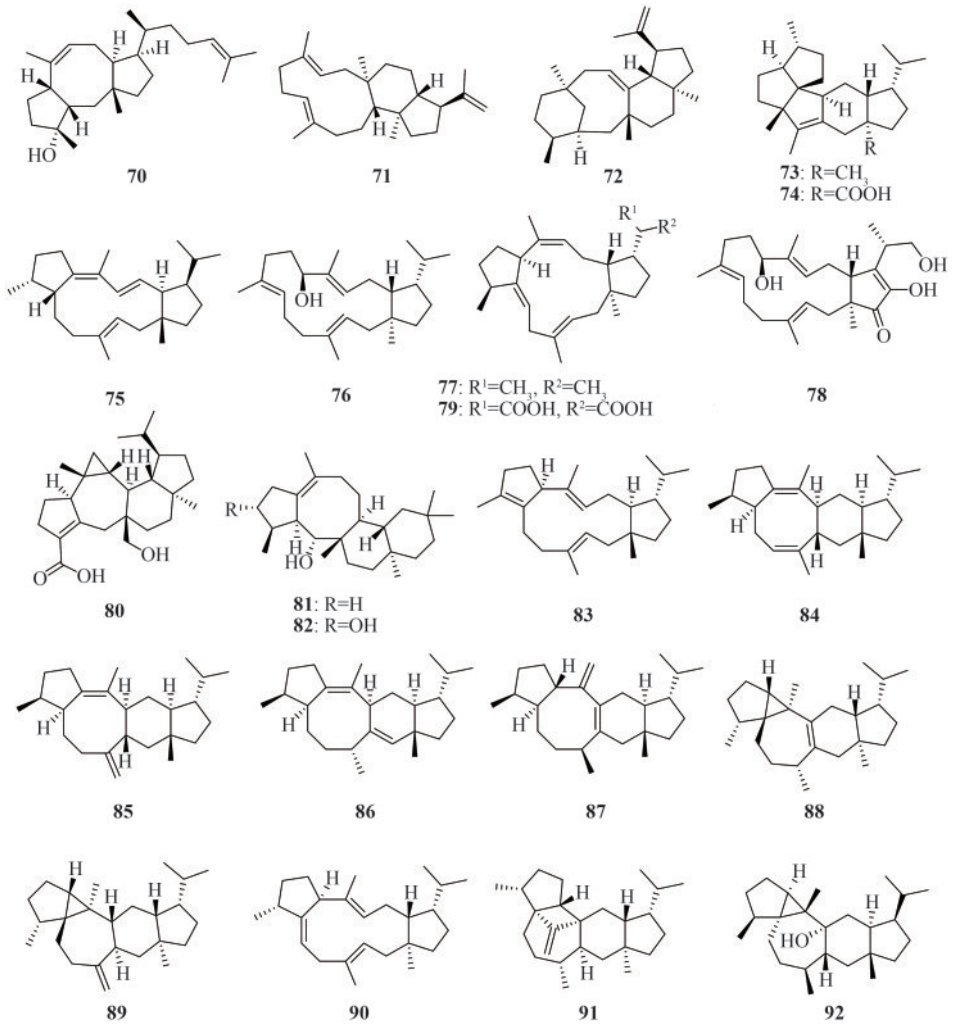


图5 经由米曲霉系统异源表达的代表性二倍半萜类化合物结构

Fig. 5 Structures of sesterterpenoids heterologously produced in the *Aspergillus oryzae* system

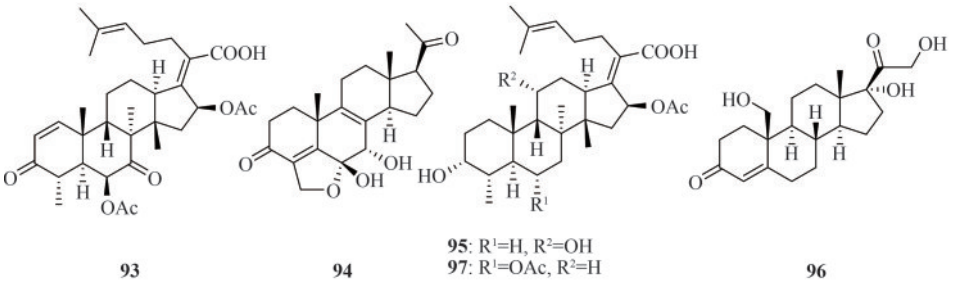


图6 经由米曲霉系统异源表达的代表性三萜及甾体类化合物结构

Fig. 6 Structures of triterpenoids/steroids heterologously produced in the *Aspergillus oryzae* system

2.5.5 杂萜类

杂萜类化合物是一类结构多样、生物活性多样的萜类化合物，其广义的结构分类可以根据结构中是否含有聚酮单元来进行[124]，而从萜类环化酶的类型则可以更加细分。本文将综合两种分类方法对米曲霉宿主异源表达该类化合物的成功案例进行分类。

（1） Ⅰ型萜烯环化酶聚酮杂萜

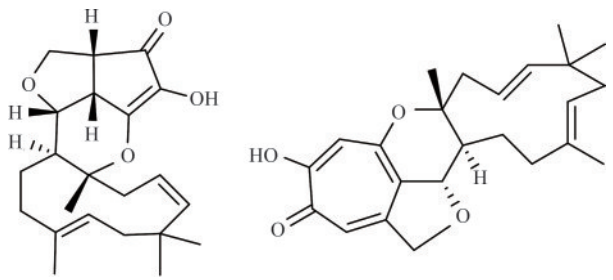
Schor 等[125] 将 *Acremonium strictum* IMI 501407 的一条杂萜基因簇在米曲霉中异源表达，

表3 米曲霉异源表达的代表性萜类化合物来源基因以及宿主信息

Tab. 3 Representative terpenoids heterologously produced in *Aspergillus oryzae*, origins of the biosynthetic genes, and hosts used for the heterologous expression

结构分类	化合物名称和编号	基因来源	表达宿主	参考文献
倍半萜	abscisic acid (**51**)	*Botrytis cinerea* SAS56	*A. oryzae* NSAR1	[92]
	trichobrasilenol (**52**)	*T. atroviride* FKI-3849	*A. oryzae* NSAR1	[93]
	aculene D (**53**)、asperaculane C(**54**)、asperaculane D(**55**)、asperaculane E(**56**)、asperaculane F(**57**)、asperaculane G (**58**)	*A. aculeatus* ATCC16872	*A. oryzae* NSAR1	[94]
	brasilane A (**59**)、brasilane D (**60**) brasilane E (**61**)	*Annulohypoxylon truncatum* CBS 140778	*A. oryzae* NSAR1	[95]
二萜	aphidicolin (**62**)	*Phoma betae* PS-13	*A. oryzae* NSAR1	[97]
	variediene (**63**)	*Emericella variecolor* NBRC 32302	*A. oryzae* NSAR1	[98]
	pleuromutilin (**64**)	*Clitopilus passeckerianus* ATCC 34646	*A. oryzae* NSAR1	[34]
		Clitopillus pseudo-pinsitus ATCC20527	*A. oryzae* NSAR1	[29]
		Clitopilus passeckerianus ATCC 34646	*A. oryzae* NSAR1	[99]
	(+)-copalol (**65**)	*Penicillium verruculosum* TPU1311 和 *Penicillium fellutanum* ATCC 48694	*A. oryzae* NSAR1	[100]
	66	*Penicillium chrysogenum* MT-12	*A. oryzae* NSAR1	[101]
	brassicicene Ⅰ (**67**)	*Pseudocercospora fijiensis* 10CR-1-24	*A. oryzae* NSAR1	[102]
	erinacine Q (**68**)	*Hericium erinaceus* yamabushitake Y2	*A. oryzae* NSPlD1	[30]
	myrothec-15(17)-en-7-ol (**69**)	*Myrothecium graminearum* ZLW0801−19	*A. oryzae* NSAR1	[103]
二倍半萜	ophiobolin F (**70**)	*Aspergillus clavatus* NRRL1	*A. oryzae* NSAR1	[106]
		Aspergillus calidoustus CBS121601	*A. oryzae* NSAR1	[107]
	stellata-2,6,19-triene (**71**)	*Emericella variecolor* NBRC32302	*A. oryzae* NSAR1	[108]
	astellifadiene (**72**)	*Emericella variecolor* NBRC32302	*A. oryzae* NSAR1	[109]
	quiannulatene (**73**) quiannulatic acid (**74**)	*Emericella variecolor* NBRC32302	*A. oryzae* NSAR1	[110]
	Bm1 (**75**)、Bm3 (**76**) Pb1 (**77**)	*Bipolaris maydis* ATCC48331 *Phoma betae* PS-13	*A. oryzae* NSAR1	[111]
	(−)-terpestacin (**78**)	*Bipolaris maydis* C5	*A. oryzae* NSAR1	[112]
	betaestacin Ⅱ (**79**)	*Phoma betae* PS-13	*A. oryzae* NSAR1	[113]
	asperterpenoid A (**80**)	*Talaromyces wortmannii* ATCC 26942	*A. oryzae* NSAR1	[114]
	asperterpenol A(**81**)、asperterpenol B (**82**)	*Aspergillus calidoustus* CBS121601	*A. oryzae* NSAR1	[115]
	aspergiltriene A (**83**)	*Aspergillus ustus* 094102	*A. oryzae* NSAR1	[116]
	aspergildiene A(**84**)、aspergildiene B(**85**)、aspergildiene C(**86**)、aspergildiene D (**87**)			
	fusoxypene A(**88**)、fusoxypene B(**89**)、fusoxypene C (**90**) (−)-astellatene (**91**)	*Fusarium oxysporum* FO14005	*A. oryzae* NSAR1	[117]
	preaspterpenacid Ⅰ (**92**)	*Aspergillus terreus* NIH 2624	*A. oryzae* NSAR1	
三萜和甾体	helvolic acid (**93**)	*Aspergillus fumigatus* Af293	*A. oryzae* NSAR1	[119]
	94	*Nodulisporium* sp. (no. 65-17-2-1)	*A. oryzae* NSAR1	[120]
	fusidic acid (**95**)	*Acremonium fusidioides* ATCC 14700	*A. oryzae* NSAR1	[121]
	96	*Thanatephorus cucumeris* NBRC 6298	*A. oryzae* NSAR1	[122]
	cephalosporin P_1 (**97**)	*Acremonium chrysogenum* ATCC 11550	*A. oryzae* NSAR1	[123]

获得了杂萜类化合物xenovulene A（**98**），发现了以前未被认识的生物合成酶类。Schotte等[126]在米曲霉中实现了xenovulene B（**99**）及其类似物的生物合成，发现了一种新的氧化环收缩重排反应机理（图7和表4）。



98 99

图7 经由米曲霉系统异源表达的代表性Ⅰ型萜烯环化酶聚酮杂萜化合物结构

Fig. 7 Structures of polyketide-terpenoid hybrids (synthesized using a class Ⅰ terpene cyclase) heterologously produced in the *Aspergillus oryzae* system

（2）Ⅱ型萜烯环化酶聚酮杂萜

Itoh等[127]将*Aspergillus fumigatus* FO-1289的聚酮合成酶和异戊烯基转移酶等在米曲霉中异源表达获得了deacetyl-pyripyropene E（**100**）。Hu等[128]在*Penicillium coprobium* PF1169基因组中发现*ppb*基因簇，通过米曲霉异源表达和底物饲喂的方法得到了化合物11-deacetyl-pyripyropene O（**101**）和deacetyl-pyripyropene A（**102**），证明了该基因簇中两个细胞色素P450单加氧酶（*ppb3-4*）的功能。Hu等[129]在*Penicillium coprobium* PF1169基因组中发现*ppb*基因簇，通过米曲霉异源表达和底物饲喂的方法合成了化合物pyripyropene A（**103**）。Matsuda等[130]将*Penicillium chrysogenum* NBRC 32030的*adr*基因簇中萜烯环化酶和四个编码裁剪酶基因异源表达至米曲霉中获得了andrastin A（**104**）。Matsuda等[131]从*Aspergillus nidulans* FGSC A4中发现了含有α-酮戊二酸依赖双加氧酶等的基因簇，将这个基因簇在米曲霉中异源表达获得了一系列austinol的生物合成前体化合物，如preaustinoid A3（**105**）。Matsuda等[26]以米曲霉为异源表达宿主，证实了anditomin（**106**）的生物合成途径由单加氧化酶、异戊烯基转移酶和萜烯环化酶调控生成。Matsuda等[132]利用米曲霉成功表达了*Aspergillus terreus* NIH 2624的P450氧化酶和异构酶等，揭示了terretonin（**107**）的生物合成途径。Matsuda等[133]在米曲霉中对*Emericella variecolor* NBRC 32302的*ctr-P450*异源表达，得到化合物citreohybridonol（**108**）。Matsuda等[134]将*Penicillium brasilianum* NBRC 6234的*prh*基因簇导入米曲霉中，得到了化合物berkeleydione（**109**），并表征了包括非血红素铁依赖双加氧酶（PrhA）的四个氧化酶。Matsuda等[135]以米曲霉为异源表达宿主，发现了内过氧化酶（NvfI）和内过氧化物异构化酶（NvfE）参与novofumigatonin（**110**）的生物合成。Bai等[136]将*Penicillium verruculosum* TPU1311的*cdm*基因簇在米曲霉中异源表达，实现了chrodrimanin B（**111**）的生物合成。汪伟光课题组[137]将*Aspergillus versicolor* 0312和*Aspergillus felis* 0260的两条杂二萜生物合成基因簇*cle*基因簇和*sre*基因簇在米曲霉中异源表达，得到了两种环化类型的杂二萜化合物chevalone E（**112**）和sartorypyrone D（**113**），近期又使用化学酶催化的方式对化合物chevalone E的A环进行结构修饰，得到了多个chevalone E的类似物[138]。Tsukada等[139]通过米曲霉实现了与杂萜类化合物**114**类似的22个化合物的生物合成。Bai等[140]将*Aspergillus* sp. TJ23的α-酮戊二酸依赖双加氧酶、短链脱氢酶和还原酶等在米曲霉中异源表达，实现了andiconin B（**115**）和andiconin D（**116**）的生物合成。Yan等[141]将*Aspergillus funiculosus* CBS 116.56中的*fnc*基因簇在米曲霉中异源表达，得到化合物funiculolide A、funiculolide B、funiculolide C、funiculolide D（**117**～**120**）。Wei等[35]以米曲霉为异源表达宿主，完成并验证了setosusin（**121**）的生物合成（图8和表4）。

（3）吲哚萜类

Tagami等[142]通过米曲霉异源表达，完成了吲哚二萜化合物paxilline（**122**）的生物合成。Tagami等[28]在米曲霉中异源表达实现了aflatrem（**123**）和β-aflatrem（**124**）的生物合成。Liu等[143]在米曲霉中完成了由*Penicillium simplicissimum* AK-40中*ptm*基因簇负责的吲哚二萜类化合物penitrem A（**125**）的生物合成。Liu等[144]在米曲霉中重构了*jan*基因簇，阐明了shearinine D（**126**）中A/B双环体系的生物合成机制。Kudo等[145]将*Pseudobotrytis terrestris* FKA-25

表4 米曲霉异源表达的代表性杂萜类化合物来源基因以及宿主信息

Tab. 4 Representative meroterpenoids heterologously produced in *Aspergillus oryzae*, origins of the biosynthetic genes, and hosts used for the heterologous expression

化合物分类	化合物名称和编号	基因来源	表达宿主	参考文献
Ⅰ型萜烯环化酶（聚酮杂萜）	xenovulene A (**98**)	*Acremonium strictum* IMI 501407	*A. oryzae* NSAR1	[125]
	xenovulene B (**99**)	*Acremonium strictum* IMI 501407	*A. oryzae* NSAR1	[126]
Ⅱ型萜烯环化酶(聚酮杂萜)	deacetyl-pyripyropene E (**100**)	*Aspergillus fumigatus* FO-1289	*A. oryzae* M-2-3	[127]
	11-deacetyl-pyripyropene O (**101**) deacetyl-pyripyropene A (**102**)	*Penicillium coprobium* PF1169	*A. oryzae* HL-1105	[128]
	pyripyropene A (**103**)	*Penicillium coprobium* PF1169	*A. oryzae* HL-1105	[129]
	andrastin A (**104**)	*Penicillium chrysogenum* NBRC 32030	*A. oryzae* NSAR1	[130]
	preaustinoid A3 (**105**)	*Aspergillus nidulans* FGSC A4	*A. oryzae* NSAR1	[131]
	anditomin (**106**)	*Emericella variecolor* NBRC 32302	*A. oryzae* NSAR1	[26]
	terretonin (**107**)	*Aspergillus terreus* NIH 2624	*A. oryzae* NSAR1	[132]
	citreohybridonol (**108**)	*Emericella variecolor* NBRC 32302	*A. oryzae* NSAR1	[133]
	berkeleydione (**109**)	*Penicillium brasilianum* NBRC 6234	*A. oryzae* NSAR1	[134]
	novofumigatonin (**110**)	*Aspergillus novofumigatus* IBT 16806	*A. oryzae* NSAR1	[135]
	chrodrimanin B (**111**)	*Penicillium verruculosum* TPU1311	*A. oryzae* NSAR1	[136]
	chevalone E (**112**)	*Aspergillus versicolor* 0312	*A. oryzae* NSAR1	[137-138]
	sartorypyrone D (**113**)	*Aspergillus felis* 0260		
	114	*Fusarium graminearum* PH-1	*A. oryzae* NSAR1	[139]
	andiconin B (**115**) andiconin D (**116**)	*Aspergillus* sp. TJ23	*A. oryzae* NSAR1	[140]
	funiculolide A、funiculolide B、funiculolide C、funiculolide D (**117～120**)	*Aspergillus funiculosus* CBS 116.56	*A. oryzae* NSAR1	[141]
	setosusin (**121**)	*Aspergillus duricaulis* CBS 481.65	*A. oryzae* NSAR1	[35]
Ⅱ型萜烯环化酶(吲哚类杂萜）	paxilline (**122**)	*Penicillium paxilli*	*A. oryzae* NSAR1	[142]
	aflatrem (**123**) β-aflatrem (**124**)	*Aspergillus flavus* NBRC 4295	*A. oryzae* NSAR1	[28]
	penitrem A (**125**)	*Penicillium simplicissimum* AK-40	*A. oryzae* NSAR1	[143]
	shearinine D (**126**)	*Penicillium janthinellum* PN2408	*A. oryzae* NSAR1	[144]
	sespendole (**127**)	*Pseudobotrytis terrestris* FKA-25	*A. oryzae* NSAR1	[145]
	lolitrem B (**128**)	*Epichloë festucae* var. *loll*	*A. oryzae* NSPlD1	[146]
其他类型杂萜	LL-Z1272β (**129**)	*Stachybotrys bisbyi* PYH05-7	*A. oryzae* NSAR1	[147]
	daurichromenic acid (**130**)	*Rhododendron dauricum*	*A. oryzae* NSAR1	[148]
	5-chlorodaurichromenic acid (**131**)	*Fusarium* sp. NBRC100844	*A. oryzae* NSAR1	
	ilicicolin A、ilicicolin B (**132**和**133**)	*Acremonium egyptiacum* F-1392	*A. oryzae* NSAR1	[149]
	biscognienyne B (**134**)	*Biscogniauxia* sp. (71-10-1-1)	*A. oryzae* NSAR1	[150]

中的六个基因导入米曲霉中进行异源表达，得到吲哚类化合物sespendole （**127**）。Jiang等以米曲霉为异源表达宿主，完成了lolitrem B （**128**）的全生物合成[146]（图9和表4）。

（4） 其他

Li等[147]在米曲霉中完成了萜类化合物关键中间体LL-Z1272β （**129**）的生物合成。Okada等[148]通过以米曲霉为异源表达宿主，完成了包括植物来源的*DCAS*基因在内的多个基因的异源表达，实现了daurichromenic acid （**130**） 的生物合成，进一步引入*Fusarium* sp. NBRC100844中的*ascD*基因，得到新化合物5-chlorodaurichromenic acid （**131**）。Araki等[149]将*Acremonium egyptiacum* F-1392中的*asc*基因簇在米曲霉中异源表达，得到化合物

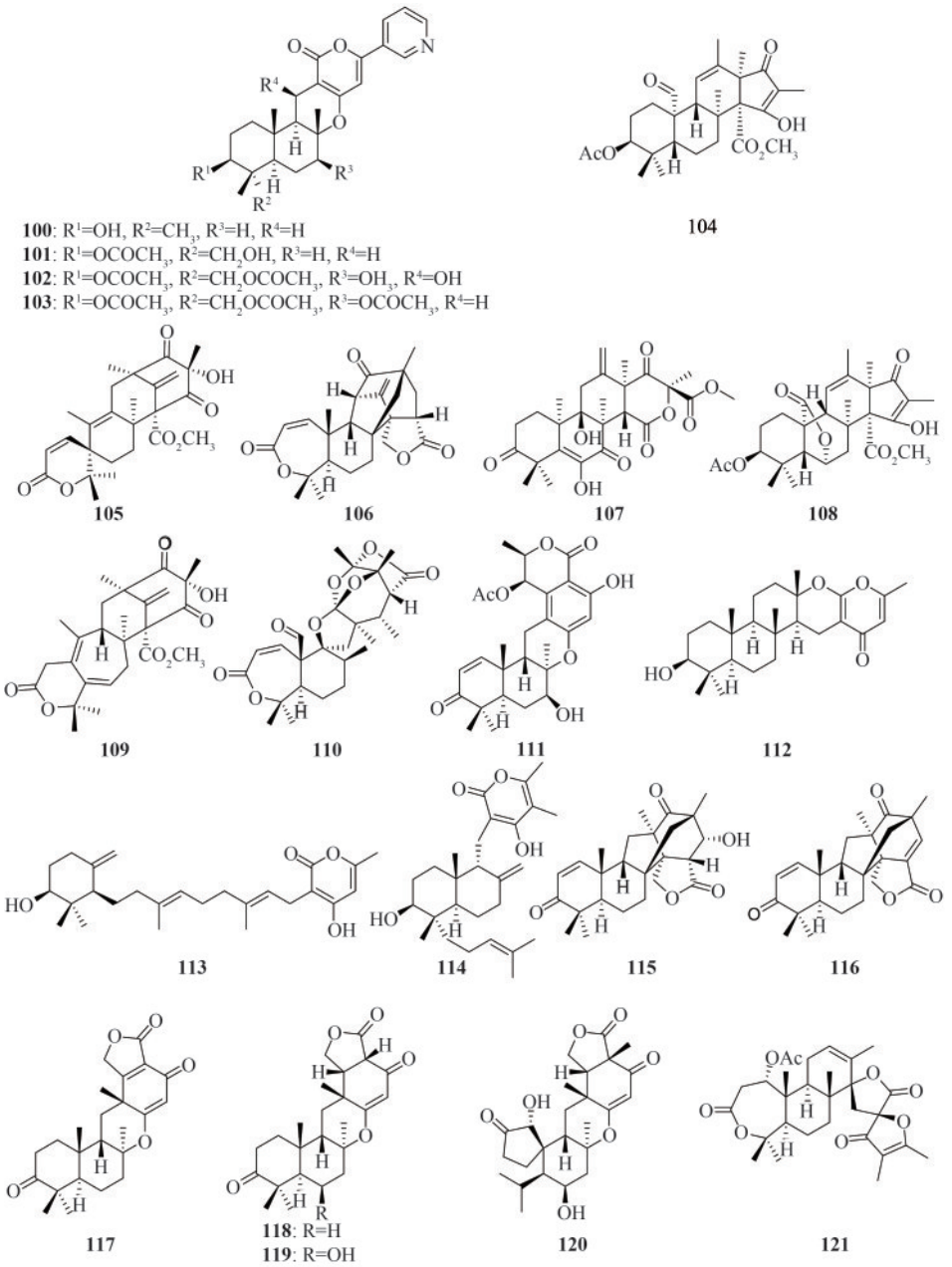


图8 经由米曲霉系统异源表达的代表性Ⅱ型萜烯环化酶聚酮杂萜化合物结构

Fig. 8 Structures of polyketide-terpenoid hybrids (synthesized using a class Ⅱ terpene cyclase) heterologously produced in the *Aspergillus oryzae* system

ilicicolin A、ilicicolin B（**132**和**133**）。Lv等[150]以米曲霉为异源表达宿主，完成了化合物biscognienyne B（**134**）的生物合成（图10和表4）。

2.6 其他类化合物

Takusagawa等在米曲霉（*A. oryzae* NSAR1）宿主中异源表达出*Neurospora crassa* JCM 19 069的*egt*基因簇，获得了化合物ergothioneine（**135**）[151]（图11）。

3 展 望

前期研究表明，米曲霉异源表达宿主不仅可以快速、高效地鉴定天然产物生物合成基因的功能，而且可以定向实现活性天然产物的基因组导

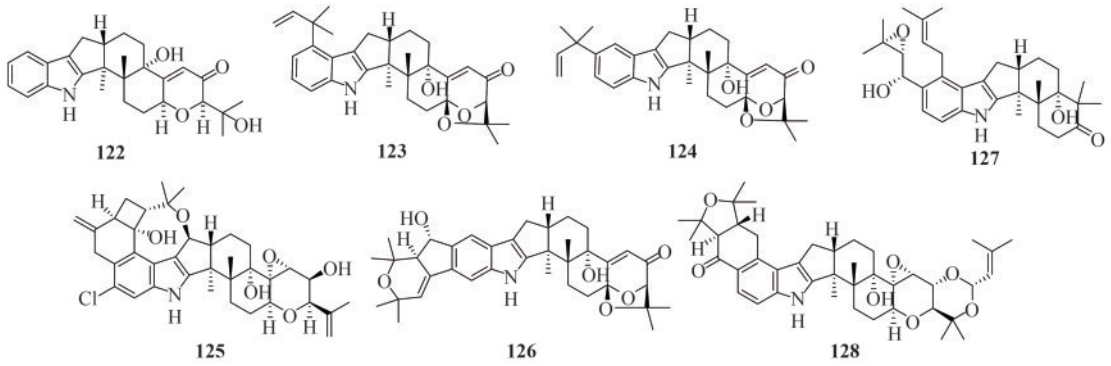


图9 经由米曲霉系统异源表达的代表性吲哚类杂萜化合物结构

Fig. 9 Structures of indole terpenoids heterologously produced in the *Aspergillus oryzae* system

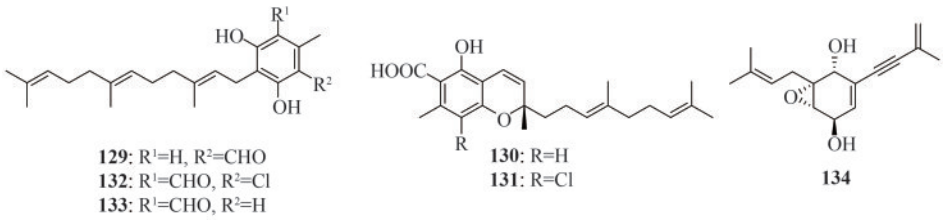


图10 经由米曲霉系统异源表达的其他类型杂萜化合物结构

Fig. 10 Structures of other meroterpenoids heterologously produced in the *Aspergillus oryzae* system

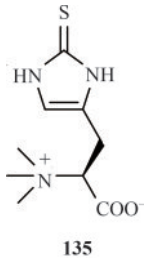


图11 经由米曲霉系统异源表达的其他类型化合物结构

Fig. 11 Structure of ergothioneine (**135**)

向挖掘，继而进一步用来实现功能基因的定向设计和改造。到目前为止，利用米曲霉已经成功表达了几乎所有文献报道的天然产物结构类型，包括聚酮化合物（PKs）、非核糖体多肽类化合物（NRPs）、核糖体肽类化合物（RiPPs）、杂合PK-NRPs、倍半萜、二萜、二倍半萜、三萜、杂萜等。其表达的天然产物生物合成基因来源不仅仅局限于丝状真菌，而且对于高等真菌、细菌乃至植物来源基因同样可以进行高效率表达。且通常情况下，与原始生产菌株相比，异源表达的化合物产量较高，例如Cox课题组通过产量计算发现，在米曲霉中进行异源表达得到化合物pleuromutilin（**64**）的产量是野生菌株的20倍左右[29, 34]。这些无不极大地促进了复杂天然产物生物合成途径的快速解析和生物合成途径重构，是一个非常理想化的天然产物合成生物学研究平台。

天然产物直接、间接为创新药物的研发应用提供了不可磨灭的贡献，青霉素、紫杉醇、洛伐他汀等可以说是大自然给予人类的馈赠，时至今日仍然有半数获批药物与天然产物相关，继续为人类健康奉献着自身的价值[152]。尽管受制于发现技术、培养方法、筛选模型和方法、资源等因素，天然创新药物的发现受到了诸多挑战[153]，然而，后基因组时代，高通量测序、宏基因组测序、单细胞测序技术等的发展为我们提供了广阔的天然产物生物合成基因素材。全新的生物活性筛选平台可以快速帮助人类提高活性先导化合物发现的效率。基因编辑、定向进化、人工智能、阿尔法-fold、蛋白质从头设计、合成生物学等技术与学科的迅猛发展和完善又给予人们更多的机遇，这些都将极大地促进天然产物的进一步研究与应用[153]。生物信息学技术以及人工智能技术的发展和应用可以帮助人们更好地进行理性设计、分析以及改造天然产物生物合成关键基因。而在这些技术和领域的发展中，异源表达技术无一不扮演着不可或缺的重要作用。前沿技术的革新和多学科交叉迅猛发展将重新定义天然药物化学研究，

这也必将会对异源表达体系提出更高的要求。安全、绿色、高效率的底盘体系也必将应运而生。

米曲霉异源表达宿主在天然产物化学研究领域已经取得了巨大的成功，但是对于该体系进行进一步完善和优化，例如开发新的转化方法、提高转化效率、缩短转化时间、寻找和设计更加高效的增强型启动子、优化宿主代谢通路等等都将是十分有必要的。米曲霉异源表达宿主的发展和完善毋庸置疑也会极大地促进更多天然产物化学研究技术的发展和革新。因此，可以预见到，在未来，不论是天然产物的基因组导向挖掘、生物合成、合成生物学乃至创新药物的研发领域，米曲霉体系都将得到更为广泛的应用。

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通讯作者：汪伟光（1984—），男，研究员，硕士生导师。研究方向为天然药物化学。

E-mail：wwg@live.cn

通讯作者：松田侑大（1988—），男，助理教授，博士生导师。研究方向为天然产物生物合成和合成生物学。

E-mail：ymatsuda@cityu.edu.hk

第一作者：董佳钰（1997—），女，硕士研究生。研究方向为真菌来源天然产物生物合成。

E-mail：785661452@qq.com