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硕士学位论文

植物内生真菌次级代谢产物的研究及化学
表观遗传学的应用初探

Studies on the Secondary Metabolites of Endophytic Fungi
and preliminary application of chemical epigenetics

黄桂红

指导教师姓名：邓贤明教授

徐庆妍副教授

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英文缩略词

缩写式	全称
NMR	Nuclear magnetic resonance (核磁共振)
MS	Mass spectrometry (质谱分析)
UV	Ultraviolet (紫外分析)
ESI-MS	Electrospray ionization mass spectrometry
IR	Infra-red (红外分析)
δ	Chemical shift (化学位移)
s	Single (单重峰)
d	Doublet (二重峰)
t	Triplet (三重峰)
q	Quartet (四重峰)
dd	Doublet of doublet (两个双重峰)
dt	Doublet of triplet (两个三重峰)
m	Multiplet (多重峰)
DEPT	Distortionless enhancement by polarization transfer
HMBC	Heteronuclear Multiple-bond correlation (碳氢远程相关)
HMQC	Heteronuclear Multiple-Quantum Coherence (异核多量子相关)
HSQC	Heteronuclear Single-Quantum Coherence (异核相关)
COSY	Correlated spectroscopy (氢氢相关)
R_f	Relative mobility (相对迁移率)
PR-18	Reversed-phase octadecyl silica gel (反相碳十八色谱柱)
TLC	Thin layer chromatography (薄层色谱)
IC ₅₀	Concentration giving 50% of maximal inhibition (半数抑制浓度)
MTT	Methyl thiazolyldiphenyl tetrazolium bromide (甲基四唑蓝)
PBS	Phosphate-buffered saline (磷酸缓冲液)
SDS	Sodium Dodecyl Sulfate (十二烷基环酸钠)
MeOH	Methanol (甲醇)

Ace	Acetone (丙酮)
EA	Ethyl acetate (乙酸乙酯)
PE	Petroleum ether (石油醚)
DMSO	Dimethyl sulfoxide (二甲基亚砜)

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摘要

特殊生境来源的真菌生存环境特殊,在适应环境的过程中形成了独特的代谢和防御系统,其次级代谢产物明显有别于普通环境下的真菌,所以特殊生境的真菌成为挖掘新次级代谢产物的新目标。其中,植物内生真菌和海洋真菌就是这类特殊生境真菌资源的重要组成部分。从植物内生真菌和海洋真菌资源中获取药物先导化合物已成为生物学、化学和药学等领域关注的焦点。真菌的许多基因在实验室培养条件下处于沉默状态。近年研究表明通过化学表观遗传操作即利用小分子化学物质抑制真菌中影响表观遗传的酶类,能激活沉默的生物合成基因,诱导真菌产生新的次级代谢产物。本论文选择特殊生境菌株作为研究对象,并且运用化学表观遗传操作,以期获得更多结构新颖且具有生物活性的次级代谢产物。

本论文采用多种柱层析方法及 1D、2D-NMR、MS、HR-MS 等波谱技术,对一株植物内生真菌次级代谢产物进行了研究,并采用组蛋白去乙酰化酶和 DNA 转移酶抑制剂对一株海洋真菌进行表观遗传修饰的调控研究。共分离、鉴定了 48 个化合物的结构,其中 17 个为新化合物。

从植物内生真菌 *Phomopsis* sp. xz-18 的发酵产物中共分离鉴定了 36 个化合物,包括 18 个细胞松弛素,4 个氧杂蒽酮类化合物,3 个氮杂吡吩类化合物和 11 个其他类型的化合物;其中 17 个为新化合物,出新率达到 47.2%。结构新颖的化合物中 9 个为细胞松弛素,其中 A8-G33b、A8-G33c、A8-G34b 骨架新颖,本论文首次报道该类结构。

从经过化学表观遗传修饰的海洋真菌,海藻共生耐盐曲霉 *Aspergillus* sp. F00785 的发酵产物中共分离鉴定了 12 个化合物,包括 4 个苯甲醛衍生物、4 个虫曲霉毒素、1 个已知萘酚类化合物和 3 个已知蒽醌类化合物 (G8-E、H1-C1、G8-D1)。其中蒽醌类化合物虽是旧结构,但在该菌中是因加了表观遗传修饰剂才产生的新化合物。

为了探究新化合物的成药价值,对新化合物进行了抗菌、抗肿瘤、抗氧化、抗酪氨酸酶和抗乙酰胆碱酯酶活性的测定。滤纸片法发现细胞松弛素 A8-G31a 对 *Bacillus pumilus* (*B.p*)、*Candida albicans* (*C.a*) 具有中等抗菌活性; A8-G31b 对 *B.p* 和 *Bacillus subtilis* (*B.s*) 具有中等抗菌活性; A8-G33c 对 *Aspergillus niger*

具有微弱活性；B7-A3b 对 *B.s*, *C.a* 具有微弱活性；A5-C11 对 *C.a*, *Staphylococcus aureus* (*S.a*) 有微弱活性；B9-D1a 对 *B.p*, *S.a* 和 *Escherichia coli* 具有较好的活性。A5-C11、A5-D41、A3-A11a 有消除自由基活性，消除率分别为 62.67%，57.52%，89.88%，其中阳性对照消除率为 95.96%。

综上，本论文从一株植物内生真菌出发成功分离鉴定了 17 个新的化合物，占所分离到的总化合物的 47.2%，说明特殊生境下的真菌（如植物内生真菌）在发现结构新颖、具有潜在生物活性的次级代谢产物方面具有更大的潜力。同时，对海洋真菌进行化学表观遗传操作后获得了三个在常规条件下未发现的次级代谢产物，展示了该策略在挖掘真菌资源新活性天然产物方面的潜在用途。

关键词：植物内生真菌；海洋真菌；次级代谢产物；化学表观遗传操作

Abstract

Fungus living in a special habitat, such as endophyte fungus and marine fungus, evolves a unique metabolism and defense system in the process of adapting to the environment. These fungi have been considered as rich sources of novel and bioactive secondary metabolites. Recent study show that chemical epigenetic modification of the fungus by reagents such as histone deacetylase (HDAC) inhibitors and/or DNA methyltransferase inhibitors could activate certain silent genes, which would lead to the production of a variety of new secondary metabolites. Employ these strategy, we have systematically investigated the natural products from the endophyte fungi *Phomopsis* sp. xz-18 and a halotolerant endogenic fungal *Aspergillus* sp. F00785 via chemical epigenetic manipulation. In this thesis, we have isolated and elucidated 48 compounds from both strains, where 17 compounds are new chemical entities.

Thirty-six compounds were isolated and elucidated from the extracts of endophyte fungi *Phomopsis* sp. xz-18, including eighteen cytochalasins, four xanthenes, three azaphilones, and eleven other types of compounds. Among them, seventeen compound, accounting for 47.2% of all obtained compounds, were novel structure. There're nine new cytochalasins, and more interestingly three of them were the first time to be reported with unique chemo-type.

Twelve compounds were isolated and elucidated from seaweed halotolerant symbiotic niger *Aspergillus* sp. F00785 via chemical epigenetic manipulation, including four benzaldehyde derivative, four asperentins derivatives, a known naphthol derivatives and three known anthraquinone derivatives (G8-E, H1-C1, G8-D1). It needs to be pointed out that these three anthaquinons were only observed under chemical epigenetic manipulation conditions, but not for untreated conditions.

These natural products were further evaluated using a panel of bioactivity assays including antimicrobial, antitumor, antioxidant, antityrosinase and anti-acetylcholinesterase tests. Compound A8-G31a showed moderate antimicrobial activity against *Bacillus pumilus*(*B.p*), *Candida albicans*(*C.a*); A8-G31b showed

moderate inhibition against *B.p* and *Bacillus subtilis* (*B.s*); A8-G33c showed weak antimicrobial activity against *Aspergillus niger*; B7-A3b showed weak antimicrobial activity against *B.s* and *C.a*; A5-C11 exhibited weak activity against *C.a* and *Staphylococcus aureus* (*S.a*); B9-D1a exhibited relative strong activity against *B.p*, *S.a* and *Escherichia coli*. A5-C11, A5-D41, A3-A11a showed relatively strong anti-free scavenging activity of DPPH.

In summary, our results of 17 new compounds out of 36 compounds in total obtained from endophyte fungi *Phomopsis* sp. xz-18 highlights the notion that fungus in special ecotope such as endophyte fungi is a rich source to discover novel and bioactive second metabolites. Epigenetic manipulation to a marine fungi activates silent gene clusters to give three anthraquinones which were not observed under untreated conditions, suggests its potential use in fully exploiting new natural products from known fungus.

Key words: endophytic fungi; marine fungi; secondary metabolite; epigenetic manipulation.

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