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特异生境真菌的抗真菌活性筛选和
八个菌株的化学成分研究

The Screening of Antifungal Activities of
Specific Environmental Fungi and Studies on Chemical
Components of 8 Fungal Strains

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摘要

真菌感染性疾病一直危害着人类的健康。在治疗真菌感染的过程中，抗真菌药物的发展过程经历了大约三个阶段：早期以天然产物药物灰黄霉素和两性霉素为主；20世纪80年代后以唑类为代表的合成药物是研究的热点；近几年系统真菌感染在临床发病率逐年增加、耐药性病原真菌不断出现的时候，echinocandins类药物的上市和 sordarins 类化合物成为热点预示着天然产物重新成为抗真菌药物研究的重点。

自然界天然产物数量巨大，种类繁多，在生命过程中起着生理调节、信号传递以及化学防御等重要作用，而且有活性的天然产物又是药物开发的重要资源。真菌是能够产生新结构天然产物的重要生物类群之一。特异环境真菌，包括海洋真菌和植物内生真菌等，由于其特殊的生存环境可以产生结构独特，骨架新颖的次生代谢产物，近年来已成为发现新药物或者先导化合物的重要资源，备受科研工作者的关注。

本文以本课题组保藏的204株海洋真菌和植物内生真菌为研究对象，进行少量发酵，对其提取物进行抗真菌（白色假丝酵母）活性的测定，挑选出60个具抗真菌活性的菌株。结合菌落形态，抗真菌活性以及提取物薄层层析显色等特征将这些菌株分为29组。选取活性最好类别不同的4个菌株AF119、AF219、AF228、AF251和4个其他菌株C56、D-20、TF-5、XZ-53进行发酵，对其化学成分进行系统的分离纯化，并利用核磁共振、质谱、紫外光谱、红外光谱、旋光和X-射线单晶衍射等方法对得到的纯化合物进行结构鉴定。共分离鉴定了3个具显著抗真菌活性的化合物和其他57个纯化合物，其中有23个新化合物。初步研究了部分化合物的生物活性。

从C56菌株固体发酵产物中分离并鉴定了8个化合物，包括6个 brefeldin A类型的大环内酯类化合物（其中1个新化合物），1个吲哚二萜化合物和1个二酮哌嗪杂合萜化合物。Brefeldin A有很强的抗菌、抗肿瘤活性，对其衍生物和抗肿瘤作用机制的研究一直都在进行，有望开发为抗肿瘤药物。后二者是一类真菌毒素，能够引起哺乳动物的震颤麻痹。

从AF119和AF251菌株固体发酵产物中分离并鉴定了13个化合物，8个对三联苯类化合物及其衍生物，1个环七肽化合物，2个含氯黄酮类化合物和2个

二苯醚类化合物。MTT 实验和流式细胞仪测定表明对联苯类化合物对肿瘤细胞增殖有较强的抑制作用，主要表现为细胞周期延缓，最终导致细胞死亡。

从 AF228 菌株固体发酵产物中活性追踪分离到 1 个抗真菌活性化合物霉酚酸，并得到其晶体结构。

从 AF219 菌株固体发酵产物中活性追踪分离到一个抗真菌活性很强的化合物 aspirochlorine，同时还分离鉴定出 9 个其他化合物，包括萜类、二酮哌嗪、苯并呋喃酮等。其中有 1 个新结构的二萜化合物。

从 D-20 菌株固体发酵产物中分离并鉴定了 5 个化合物，4 个倍半萜和 1 个化合物双酚 A。其中 2 个菖蒲二烯结构的倍半萜是新化合物。

从 XZ-53 菌株固体发酵产物中离并鉴定了 3 个化合物，2 个聚酮化合物和 1 个聚酮杂合萜化合物，其中 1 个聚酮是新化合物。

从 TF-5 菌株固体发酵产物中离并鉴定了 20 个化合物，8 个二氢异苯并吡喃酮类化合物，其中 3 个含氯的是新化合物；7 个萜类化合物，其中有 5 个新结构的倍半萜；还有其他 3 个新化合物和 2 个已知化合物。

本文研究结果表明，海洋真菌和植物内生真菌等特异环境来源的真菌能够产生抗真菌活性的天然产物和新结构的天然产物，这些天然产物是新药研发重要的先导化合物。

关键词：真菌；抗真菌活性；天然产物；结构鉴定

Abstract

Fungal diseases have long endangered mankind's health. During the process of curing fungal infections, the development of antifungal agents experienced three stages. In early days natural product medicines griseofulvin and amphotericin B are the major. Since 1980's, synthetic azoles is the major class of antifungal agents in clinic. The last few years, new antifungal agents echinocandins were used and sordarins were studied to treat systemic fungal infections. This indicated that natural products rebecame an important resource to get new antifungal agents.

The amount of natural products is very huge and category is numerous. They played not only a leading actor in the fields of physiological regulation, chemical defense and signal transduction for the organism, but also a vital role in the discovery of new pharmacy. Specific environmental fungi, including marine fungi and endophytic fungi have been to a new resource to find leading compounds because they can produce new natural products with novel skeleton for their special living environment. Specific environmental fungi have drawn much researchers' concern.

In this study 204 marine fungi and endophytic fungi were fermented on a small scale. Their organic extracts were used to screen for antifungal (*Candida albicans*) activity. This yielded 60 active strains which were devided into 29 groups based on their colony morphology, antifungal activity and thin layer chromatography characteristic. The four strains, with the best activity, named AF119, AF219, AF228 and AF251 respectively, were selected for further investigation of natural products. These were later fermented on a large scale. Four other strains named C56, D-20, TF-5 and XZ-53 were also investigated. In total, 3 antifungal compounds and 57 other natural products including 23 new ones were isolated from these selected strains. Their structures were elucidated by NMR spectroscopic data, mass spectroscopic, UV, IR, rotational and X-ray single crystal diffraction. Some were assayed for their bioactivities.

Eight compounds were isolated and elucidated from the strain C56, fermented in solid medium. These included 6 macrolides (one new compound), 1 indolediterpene and 1 indolediketopyperazin. Brefeldin A, one of the macrolides, exhibited strong antimicrobial and antitumor activity. The two other

compounds are mycotoxins.

Thirteen compounds were isolated and elucidated from the strain AF119 and AF251 fermented in solid medium. These included 8 *p*-terphenyl compounds and their derivatives, 1 cyclic peptide, 2 flavones and 2 diphenyl ethers. MTT assay and flow cytometry of 8 *p*-terphenyl compounds antitumor activity showed that *p*-terphenyls exhibits cytotoxicity.

Bioassay-guided isolation of the strain AF228 fermented in solid medium yield the antifungal compound, mycophenolic acid.

From the strain AF219 cultured by solid fermentation, 10 compounds were isolated and elucidated. These included terpenes, benzofuranone and indole-diketopyperazins, etc. Bioassay-guided isolation of the strain yielded, notably the compound aspirochlorine which exhibited very strong antifungal activity.

Five compounds were isolated and elucidated from the strain D-20 cultured in solid medium. These included 4 sesquiterpenes and the compound bisphenol A. Two sesquiterpenes with acoradiene structure were obtained as new compounds.

There polyketide compounds, including 1 new compound, were isolated and elucidated from the strain XZ-53.

Twenty compounds, including 8 dihydroisocoumarins, 7 terpenes and 5 other compounds, were isolated and elucidated from the strain TF-5 fermented in solid medium. Among these, there were 11 new compounds.

In conclusion, our study showed that specific environmental fungi, such as marine fungi and endophytic fungi can produce antifungal natural products and other natural products with new structure. These compounds will be important leads in the discovery of new agents.

Key word: Fungi; Antifungal activity; Natural product; Structure elucidation.

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