学校编码：10384　　分类号_____密级 _____

学号：20120051403136　　UDC _____

厦门大学

博 士 学 位 论 文

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

刘 韶 松

指导教师姓名：　沈 月 毛 教授

专 业 名 称：　微 生 物 学

论文提交日期：　2009 年 6 月 16 日

论文答辩日期：　2009 年 7 月 4 日

学位授予日期：　2009 年 月 日

答辩委员会主席：苏文金 教授

评 阅 人：_____

2009 年 7 月

厦门大学学位论文原创性声明

本人呈交的学位论文是本人在导师指导下,独立完成的研究成果。本人在论文写作中参考其他个人或集体已经发表的研究成果，均在文中以适当方式明确标明，并符合法律规范和《厦门大学研究生学术活动规范（试行）》。

另外，该学位论文为（　　）课题（组）的研究成果，获得（　　）课题（组）经费或实验室的资助，在（　　）实验室完成。（请在以上括号内填写课题或课题组负责人或实验室名称，未有此项声明内容的，可以不作特别声明。）

声明人（签名）：

年　　月　　日

厦门大学学位论文著作权使用声明

本人同意厦门大学根据《中华人民共和国学位条例暂行实施办法》等规定保留和使用此学位论文，并向主管部门或其指定机构送交学位论文（包括纸质版和电子版），允许学位论文进入厦门大学图书馆及其数据库被查阅、借阅。本人同意厦门大学将学位论文加入全国博士、硕士学位论文共建单位数据库进行检索，将学位论文的标题和摘要汇编出版，采用影印、缩印或者其它方式合理复制学位论文。

本学位论文属于：

（　　）1.经厦门大学保密委员会审查核定的保密学位论文，于　　年　　月　　日解密，解密后适用上述授权。

（　　）2.不保密，适用上述授权。

（请在以上相应括号内打"√"或填上相应内容。保密学位论文应是已经厦门大学保密委员会审定过的学位论文，未经厦门大学保密委员会审定的学位论文均为公开学位论文。此声明栏不填写的，默认为公开学位论文，均适用上述授权。）

声明人（签名）：

年　　月　　日

目　　录

CONTENTS

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. Since1980'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.