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硕 士 学 位 论 文

红树植物内表生放线菌的分离、活性筛选和
次级代谢产物研究

Study on the Isolation, Identification, Bioactivity and
Secondary Metabolites of Mangrove Actinomycetes

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摘要

天然产物是药物的重要来源。放线菌(*Actinomycetes*)在产生抗生素方面具有独特的优势,大约75%的抗生素都是由放线菌产生,从植物内生放线菌的次级代谢产物中,可以分离到许多结构新颖,生物活性多样的化合物。红树植物生长于海岸潮间带,其独特的生存环境赋予它丰富的微生物资源,是当前倍受重视的药用新资源。

本论文对采集自福建省漳州市浮宫镇九龙江口、福建省厦门市集美和海沧三个地点的两种红树植物(秋茄和桐花树)的叶部和根部进行内表生放线菌的分离,共得到 55 株放线菌。

以枯草芽孢杆菌(*Bacillus subtilis*)、大肠杆菌(*Escherichia coli*)、金黄色葡萄球菌(*Staphylococcus aureus*)、短小芽孢杆菌(*Bacillus pumilus*)、白色假丝酵母(*Candida albicans*)和黑曲霉(*Aspergillus niger*)为指示菌,采用双层琼脂扩散法对这些菌株进行抗菌活性检测,共筛选得到活性菌株 26 株,占总菌株数的 47.3%,其中主要是抗革兰氏阳性细菌的活性;采用 MTT 法对其体外抗肿瘤活性进行检测,发现 5 株放线菌对 Hela (人子宫癌细胞)和 HepG2 (人肝癌细胞)具有相对较高抑制作用,占总菌株数的 9.1%。

通过 16S rDNA 的序列分析,对所分离菌株进行了分子鉴定,结果表明只有一株放线菌属于拟诺卡氏菌属(*Nocardiosis*),其他均为链霉菌属(*Streptomyces*)。通过对分离菌株进行 AHBA 引物筛选,有 6 株放线菌筛选结果为阳性,表明这 6 株菌的 DNA 中可能含有 AHBA (3-氨基-5-羟基苯甲酸)合成酶基因,其中包括拟诺卡氏菌。

本论文对该株拟诺卡氏菌(3号菌)和另一株具有较高抗菌和抗肿瘤活性的链霉菌(18号菌)的次级代谢产物进行了初步的研究。从3号菌株的次级代谢产物中分离并鉴定了13个化合物,其中3个为新化合物(3C、3Y-10),属于吡喃酮类化合物;10个为已知结构的化合物(3A、3Y-5、3Y-7、3Y-14、3L-A、3L-M、3L-G、3Y-18、3Y-19、3Y-20):3A、3Y-5、3Y-7和3Y-14为色氨酸的衍生物;3L-A为邻氨基苯甲酸甘油酯;3L-M为水杨酰胺类化合物;3L-G为乙酸甘油酯;3Y-18为乙酰酪胺;3Y-19和3Y-20为环二肽。从18号菌株的次级

代谢产物中分离并鉴定了 4 个已知结构的化合物 (18-C、18-F、18-E、18-G)，分别为对羟基苯乙酸甲酯、 α -酮戊二酸二甲酯、2,6-二脱氧吡喃糖苷、2,6-二脱氧吡喃糖。对分离到的新化合物 3C 的抗菌活性及抗肿瘤活性进行了研究，在每片滤纸片含有 50 μ g 样品 (滤纸片法) 时对 5 种指示菌没有显示出抗菌活性；抗肿瘤实验结果显示，3C 对 HeLa 细胞只有轻微的抑制作用，在 10 μ g/mL 和 20 μ g/mL 两种浓度时的抑制率分别为 2.73% 和 7.39%。

本文结果表明，红树林放线菌中蕴藏着较丰富的抗菌和抗肿瘤活性物质产生菌，同时也蕴藏着较丰富的结构新颖的活性物质，是开发抗菌抗肿瘤等药物的潜在资源。

关键词：红树植物；内表生放线菌；生物活性；次级代谢产物

Abstract

Nature products are important source of Medicine. Actinomycetes have a distinguished advantage in the field of antibiotics: about 75% of antibiotics are produced by actinomycetes, from the secondary metabolites of endophytic actinomycetes, a lot of compounds with new structures and great bioactive diversities could be found. Mangrove plants as important new resources for potential pharmaceutical because of their ecosystems that straddled the land and the sea, from freshwater to seawater, often with distinct microorganism species, have gradually attracted more and more attention of natural product chemists.

From the leaves and roots of two mangrove plants (*Kandelia candel* and *Aegiceras corniculatum*) which collected from Fugong, Jimei, Haicang in Fujian Province, 55 mangrove actinomycetes (endophytic or epiphytic) were isolated.

In the investigation of antimicrobial activities, 26 strains (about 47.3% of the total isolates) which showed antimicrobial activities against one or more sensitive microbes (*Bacillus subtilis*, *Bacillus pumilus*, *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Aspergillus niger*) were screened out by using the agar diffusion assay. Using the method of MTT (Methyl Thiazolyltetrazolium), the isolates were screening for antitumor strains in vitro. The results showed that 5 strains (9.1% of the total isolates) could inhibit the growth of Hela and HepG2 tumor cell lines.

The identification of the isolates by 16S rDNA assay indicated that only one strain belonged to *Nocardioopsis* while all the others belonged to *streptomyces*. The diversity of AHBA synthase gene was investigated using a pair of primers, AHBA R1-AHBA F. The KS domain of each isolate was amplified. 6 strains could be amplified and amplification products displayed 700bp DNA bands, indicated DNA of these strains might have AHBA synthase gene.

The strain *Nocardioopsis* sp. (No.3) and another bioactive strain

streptomyces sp. (No.18) were selected to study their secondary metabolites. 13 compounds were isolated from strain No.3, including 3 new compounds (3C, 3Y-10) which were pyranones and 10 known compounds (3A, 3Y-5, 3Y-7, 3Y-14, 3L-A, 3L-M, 3L-G, 3Y-18, 3Y-19, 3Y-20). Within the 10 known compounds, 3A, 3Y-5, 3Y-7, 3Y-14 were derivatives of tryptophan, 3L-A was glycerol 2-aminobenzoate, 3L-M was hydroxybenzamide, 3L-G was glycerol acetate, 3Y-18 was acetyltyramine, 3Y-19 and 3Y-20 were Cyclic dipeptides. From strain No.18, 4 known compounds (18-C, 18-F, 18-E, 18-G) were isolated, which were Methyl *p*-hydroxyphenylacetate, Dimethyl α -ketoglutarate, 2,6-dideoxypyranoside, 2,6-dideoxypyranose, respectively. In the later investigation of the antimicrobial and antitumor bioactivities, the new compound 3C showed no antimicrobial bioactivity at 50 μ g/disk but weak antitumor bioactivity on Hela cell, the inhibition ratios were 2.73% and 7.39% when the concentrations of 3C were 10 μ g/mL and 20 μ g/mL.

Our study indicated that mangrove plants included numbers of potent actinomycetes with antitumor and antimicrobial bioactivity, and that they were reliable resources for novel metabolites with antitumor and antimicrobial bioactivity at the same time.

Key words: mangrove plants; endophytic/epiphytic actinomycetes; bioactivity; secondary metabolites.

1. 前言

1.1 放线菌与天然产物

天然产物在药物的发现和发展的过程中发挥了重要的作用^[1]。据美国调查机构自 1983-1994 年对每年新应用的新药的调查报告显示,60-75%的抗癌药和抗感染药最初都来源于天然产物^[2]。

微生物起源久远、生境丰富,次级代谢产物的多样性,使其成为天然产物的主要来源之一。而在微生物中,放线菌是天然产物研究的重要类群。

放线菌属于需氧的革兰氏阳性细菌,在形态、生理和生化上都有别于其他的革兰氏阳性细菌,其 GC 含量占整个碱基总数的 69-78%,培养时产生大量的基内菌丝和气生菌丝。目前,在《伯杰氏系统细菌学手册》第四卷中,放线菌归属于细菌域,厚壁菌门,放线菌纲,放线菌目,包括 10 个亚目,31 个科,140 多个属。

原核微生物 53 个门中,只有其中的 5 个门能产生各种生物活性化合物。在这 5 个门中,从放线菌门的放线菌目(Actinomycetales)的微生物(通常称为放线菌)中就发现了大约 7000 个具有抗菌、抗肿瘤等活性的化合物。放线菌(Actinomycetes)具有无可比拟的生物合成能力,在已知的抗生素、抗肿瘤药物、酶抑制剂和免疫抑制剂等有生物活性的次级代谢产物中有一半都来自放线菌^[3, 4]。放线菌在产生抗生素方面具有独特的优势,大约 75%的抗生素是由放线菌产生,而其中的 75%又是由链霉菌属(*Streptomyces*)产生^[5]。

1943 年,美国科学家 Waksman 从放线菌中发现链霉素,给当时被视为不治之症的结核病人带来了福音,造就了放线菌的研究和抗生素的蓬勃发展。后来又陆续从放线菌中发现了新霉素(1949 年)、土霉素(1950 年)、红霉素(1952 年)、四环素(1953 年)等^[6]。对放线菌研究使得更多的有用的天然产物不断被发现和报道,从诺卡氏菌属中分离到的 Brasilidine A, 属于吡啶生物碱,带有独特的共轭三烯和乙氧单元,在体外表现出抗多种肿瘤细胞的活性,该化合物还具有抗革兰氏阳性菌和多种真菌的活性,尤其是对曲霉(*Aspergillus niger*)和分枝菌属(*Mycobacterium smegmatis*)表现出强抗菌活性,其 MIC 分别是 0.39 $\mu\text{g/ml}$ 和 0.78 $\mu\text{g/ml}$ ^[7, 8]。

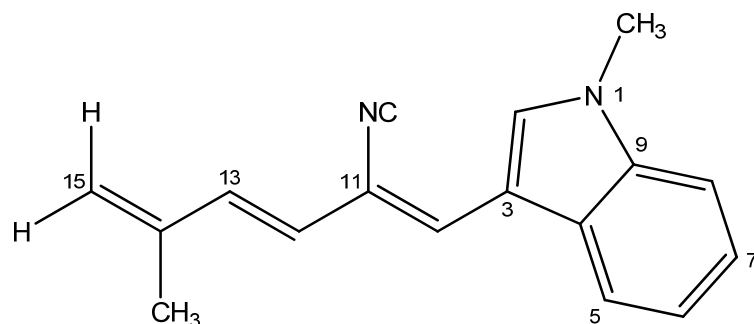
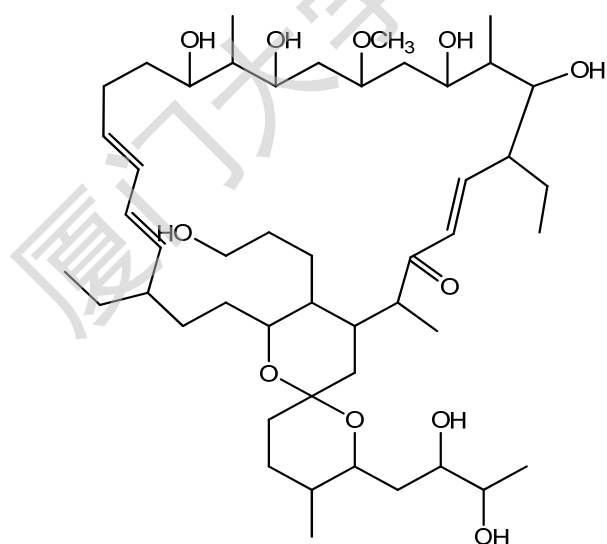
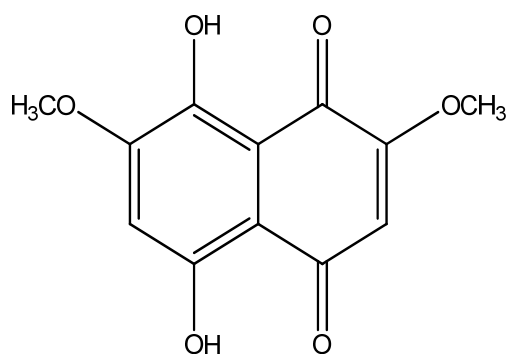


图1-1 化合物Braslidine A 的结构

Fig.1-1 The structure of Braslidine A

资料来源: Junichi Kobayashi, Masashi Tsuda, Akira Nemoto et al. Braslidine A, a New Cytotoxic Isonitrile-Containing Indole Alkaloid from the Actinomycete *Nocardia brasiliensis*. [J]. J. Nat. Prod., 1997,60: 719-720.

从放线菌 *Actinomycete* sp.Y8521050 中分离得到寡霉素族的 **Maclafungin**, 该化合物对丝状真菌如镰刀菌的活性强于酵母菌如白色假丝酵母^[9]; 从放线菌 HILY-8620959 中分离得到的 **Mathemycin B** 对多种植物病原真菌有一定的抗菌活性^[10]; 从一株不产孢的链霉菌 *Streptomyces* sp. No.12396 中分离得到的 **5,8-dihydroxy-2,7-dimethoxy-1,4-naphthoquinone**, 是一种具有一定抗革兰氏阳性细菌和真菌活性的蒽醌类化合物^[11]。

Maclafungin^a

5, 8-dihydroxy-2, 7-dimethoxy-

1, 4-naphthoquinone^b

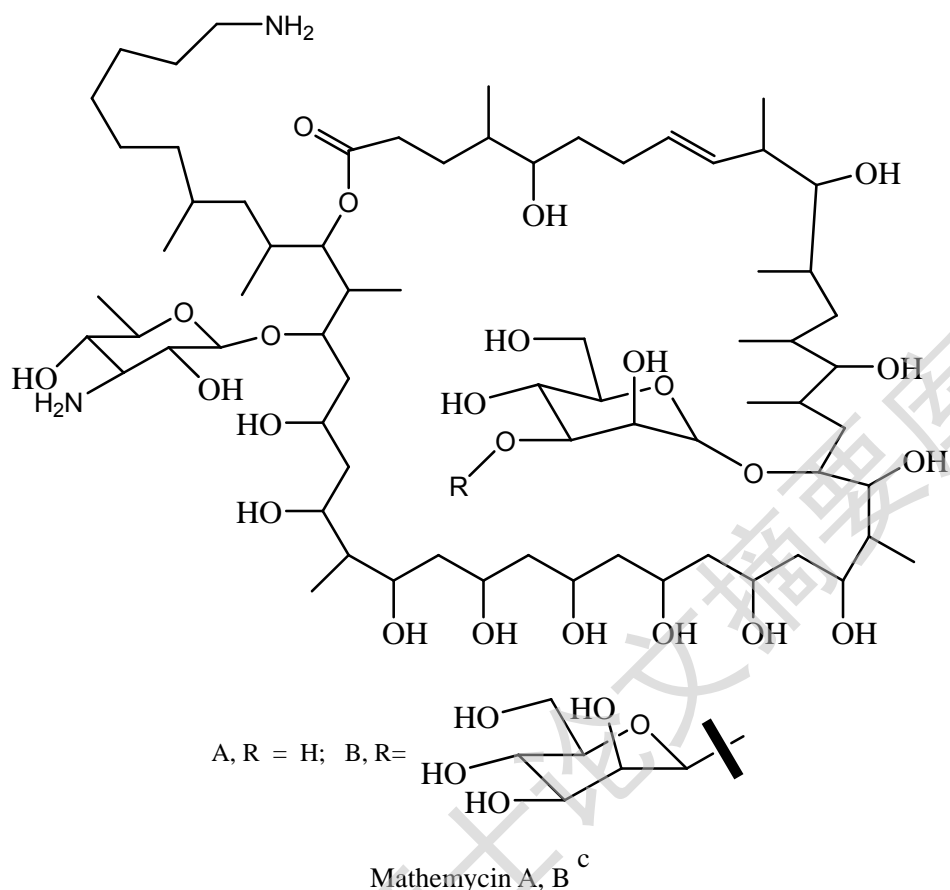


图 1-2 放线菌产生的具有抗菌活性的化合物结构

Fig.1-2 The structures of antibiotic compounds produced by actinomycetes

资料来源: a: Tripikumar Mukhopadhyay, S.R.Nadkani, M.V.Patel et al. Maclafungin, a New Antifungal Macrocyclic Lactone from Actinomycete sp.Y-8521050.[J]. Tetrahedron, 1998, 54 : 13621-13628.; b:Nancy Nichols and Bonifacy . The Structures of Two Naphthoquinone Pigments from an Actinomycete'.[J]. Wieclawek Gerber and Wieclawek, 1965, 31:1496-1498.; c:Triptikumar Mukhopadhyay, S. R. Nadkarni, R. G. Bhat et al. Mathemycin B, a New Antifungal Macrolactone from Actinomycete Species HIL Y-8620959.[J]. J. Nat. Prod. 1999, 62: 889-890.

放线菌除了能够产生具有抗菌和抗肿瘤活性的物质外,还可以产生一些非抗生素物质(nonantibiotic)。从放线菌 *Kitasatosporia kifunense* No. 9482 中分离得到的 Kifunensine, 能抑制 α -甘露糖苷酶, 可能具有免疫调节的功能^[12]; Pteridic Acid A 和 Pteridic Acid B, 则有类似植物生长因子的功能^[13]。

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