

微生物学报 *Acta Microbiologica Sinica*
54(5): 552–562; 4 May 2014
ISSN 0001–6209; CN 11–1995/Q
http://journals.im.ac.cn/actamicrocn
doi: 10.13343/j.cnki.wsxb.2014.05.010

南海局部海洋沉积物中真菌多样性及产酶活性

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摘要 【目的】从 11 份南海海洋沉积物中分离耐盐真菌, 并对其物种多样性及产酶活性进行研究。【方法】利用平板涂布法分离耐盐真菌, 基于形态学和 ITS 序列的系统进化研究耐盐真菌多样性; 利用 6 种筛选培养基对耐盐真菌进行产酶活性筛选。【结果】分离得到 1689 株耐盐真菌, 共 41 个形态种。形态学和 ITS 序列分析表明, 这些真菌归于 15 个属, 其中曲霉属 (*Aspergillus*) 和青霉属 (*Penicillium*) 为优势菌群。对已测序的 41 株耐盐真菌的产酶活性研究表明 8 株产纤维素酶, 9 株产淀粉酶, 5 株产复合酶, 16 株产蛋白酶, 3 株产脂肪酶, 未发现产壳聚糖酶的菌株, 其中 *Acrodontium* sp. 8m 和 *Aspergillus* sp. 86b 产复合酶的活性相对较高, 而 *Penicillium* sp. 41m 产蛋白酶的活性相对较高。【结论】南海局部海洋沉积物中耐盐真菌丰富, 多数菌株具有产酶活性。

关键词: 海洋沉积物, 耐盐真菌, 物种多样性, 酶活性

中图分类号: Q936 **文章编号:** 0001-6209(2014)05-0552-11

海洋沉积物是在漫长的地质变化过程中, 由陆地河流、大气输入和人类活动中落入海底的物质组成, 主要包括软泥沙、灰尘、动植物的遗骸、宇宙尘埃等^[1]。海洋沉积物不同于淡水沉积物、土壤等陆地环境, 又与海水水体环境相对独立^[2]。在海洋沉积物中存在独特的适应高盐、高压、缺氧及低温等极端环境的细菌、古菌、放线菌及真菌等。这些极端微生物 (Extremophiles) 群体在生命进化的历程中, 由于适应局部极端环境的结果, 形成独特的群落结构、遗传特性、生理机制和代谢类型, 因具有重要的科学和应用价值而成为世界生物学研究的热点之一。近年

来, 有关海洋沉积物微生物的研究更多集中在细菌、古菌和放线菌方面^[3–5]; 只有少量的工作集中在耐盐真菌方面, 却获得许多产酶、产抗生素类等活性物质的菌株^[6–8]。这些研究工作充分表明海洋沉积物中微生物资源丰富, 并具有广阔的应用前景。

南海位于太平洋沿岸, 毗邻西太平洋暖池区, 是我国面积最大、最深的边缘海, 珠江、湄公河、湄南河等三大径流常年为南海输送陆源物质, 因其微生物数量丰富, 是研究海洋微生物的理想区域之一^[9–10]。南海海洋真菌不仅在维持海洋生态系统的稳定性及参与 C、N、P 等元素的地球化学循环中

基金项目: 国家“973 项目”(2010CB833801); 国家自然科学基金(31100017); 陕西省青年科技新星计划项目(2013KJXX-76)

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收稿日期: 2013-08-14; 修回日期: 2013-11-30

发挥重要作用,而且在资源开发利用方面也有重要的应用价值^[11]。Lai 等^[12]利用免培养技术从南海沉积物中发现多个真菌属,如 *Phoma*, *Malassezia*, *Cylindrocarpon*, *Hortaea*, *Pichia*, *Aspergillus* 和 *Candida*。Zhang 等^[13]从南海深海沉积物中分离到 27 个真菌形态种,56% 的菌株具有抗菌活性。虽然南海蕴藏着丰富多样的真菌资源,但我国对该海域耐盐真菌的报道仍然较少^[14-16],限制了对南海真菌资源的进一步开发利用。

本项目对南海局部海洋沉积物的耐盐真菌多样

性及产酶活性菌株进行研究,旨在发掘具有产酶活性的微生物资源,为海洋沉积物中耐盐真菌的进一步开发利用奠定理论基础。

1 材料和方法

1.1 材料

1.1.1 材料来源:从南海局部海域采集得到 11 份海洋沉积物样品(表 1),采集后放入无菌试管内,4℃ 冷藏备用。

表 1. 南海局部海洋沉积物样品基本情况

Table 1. Basic data of marine sediment samples of South China Sea

sample	date	longitude	latitude	depth/m	sediment type
1#	2011.09.04	120°0.250'E	20°22.971'N	3536.00	soil
2#	2011.09.02	119°31.949'E	18°44.606'N	3415.00	soil
3#	2011.09.08	116°58.573'E	18°58.472'N	3763.00	soil
4#	2011.09.08	116°43.681'E	20°14.109'N	/	sandy soil
5#	2011.08.29	112°0.124'E	18°0.541'N	2448.32	soil
6#	2011.09.08	117°58.223'E	19°0.368'N	3739.00	soil
7#	2011.09.06	116°30.202'E	22°29.355'N	35.00	sandy soil
8#	2011.08.29	112°30.203'E	18°1.654'N	2439.00	soil
9#	2011.09.05	119°19.896'E	19°41.569'N	2918.00	soil
10#	2011.09.05	119°31.949'E	18°2.114'N	/	soil
11#	2011.09.02	118°30.989'E	18°5.255'N	3928.00	soil

1.1.2 培养基: (1) 分离培养基: ①高盐察氏培养基(g/L): NaNO₃ 2.0, KCl 0.5, NaCl 60.0, 蔗糖 30.0, MgSO₄·7H₂O 0.5, FeSO₄·7H₂O 0.01, KH₂PO₄ 1.0, 琼脂 15.0, 海盐 30.0; ②YPD 培养基(g/L): 酵母提取物 5.0, 蛋白胨 10.0, 葡萄糖 20.0, 琼脂 15.0, 海盐 30.0; ③玉米粉琼脂培养基(g/L): 玉米粉 60.0, 琼脂 15.0, 海盐 30.0; ④MEA 培养基(g/L): 麦芽浸粉 20.0, 蛋白胨 1.0, 葡萄糖 15.0, 琼脂 20.0, 海盐 30.0, pH 6.0; ⑤改良 PDA 培养基(g/L): 去皮土豆 200.0, 葡萄糖 20.0, 维生素 B₁ 0.01, KH₂PO₄ 5.0, MgSO₄·7H₂O 3.0, 琼脂 12.0, 海盐 30.0; ⑥沙氏培养基(g/L): 葡萄糖 40.0, 蛋白胨 10.0, 琼脂 15.0, 海盐 30.0, pH 4.0-6.0; ⑦豆芽汁培养基(g/L): 黄豆芽 100.0, 葡萄糖 20.0, 琼脂 15.0, 海盐 30.0。 (2) 产酶筛选培养基: ①纤维素酶筛选培养基(g/L)^[17]: (NH₄)₂SO₄ 2.0, MgSO₄·7H₂O 0.5, K₂HPO₄ 1.0, NaCl 0.5, 羧甲基纤维素钠 2.0, 刚果红 0.4, 海盐 30.0, 琼脂 22.0; ②淀粉酶筛

选培养基(g/L)^[18]: 淀粉 20.0, KCl 0.5, NaNO₃ 2.0, K₃PO₄ 1.0, MgSO₄·7H₂O 0.5, 海盐 30.0, 琼脂 18.0; ③复合酶筛选培养基(g/L)^[19]: NaNO₃ 3.0, KCl 0.5, FeSO₄·7H₂O 0.01, K₂HPO₄ 1.0, MgSO₄·7H₂O 0.5, 蔗糖 30.0, 干酪素 10.0, 海盐 30.0, 琼脂 15.0, pH 6.0-6.5; ④蛋白酶筛选培养基(g/L)^[20]: 蛋白胨 10.0, 葡萄糖 1.0, NaCl 5.0, CaCl₂ 0.1, 酪氨酸 0.1, 干酪素 5.0, 海盐 30.0, 琼脂 22.0, pH 7.0; ⑤脂肪酶筛选培养基(g/L)^[21]: K₂HPO₄ 2.0, MgSO₄·7H₂O 0.5, 2% PVA 乳化液 20.0 mL, 酵母粉 2.0, 蛋白胨 10.0, 海盐 30.0, 琼脂 27.0。灭好菌后冷却到 60℃ 加 10.0 mL 灭过菌的 0.2% 罗丹明 B 溶液; ⑥壳聚糖酶筛选培养基(g/L)^[22]: 壳聚糖 10.0, (NH₄)₂SO₄ 2.5, MgSO₄·7H₂O 0.25, K₂HPO₄ 0.02, NaCl 4.0, CaCO₃ 6.0, 海盐 30.0, 琼脂 20.0, pH 6.0。 1.1.3 主要仪器和设备: 组织研磨机(MM400 型, 德国 Retsch 公司); 高级研究显微镜(E600 型, 日本尼康公司); 高速冷冻离心机(5424R 型, 德国艾本

德股份公司); PCR 扩增仪(MyCycler 型, 美国伯乐公司); 电泳仪(EPS300 型, 上海天能科技有限公司); 涡旋混合仪(Vortex Genius 3, 德国 IKA 集团); 凝胶成像系统(英国 UVItec 公司)。

1.2 菌株分离及保藏

1.2.1 样品处理: 称取 1.0 g 海洋沉积物样品, 在无菌操作下加入装有 9 mL 无菌水的三角瓶中, 摇匀, 预培养 3 d 备用。

1.2.2 菌株分离培养: 采用平板涂布法对 11 份海洋沉积物样品进行分离, 分别取 100 μ L 预培养后的菌悬液涂布在分离培养基上, 置于 28 $^{\circ}$ C 培养, 对平板上生长的不同形态的菌落进行编号并观察记录。用接种针挑取少许菌丝培养, 并纯化至单菌落, 并将纯化菌株接种至斜面, 4 $^{\circ}$ C 保藏备用。

1.3 菌株的分类鉴定

采用形态学及分子生物学相结合的方法进行初步鉴定。根据菌落特征、菌丝、孢子及产孢结构等进行形态学鉴定。挑取不同形态种并利用改进的 CTAB 法^[23] 提取基因组 DNA, 通过 PCR 扩增 ITS-rDNA 基因片段。引物序列 ITS1 (5'-TCCGTAGGTGAACCTGCGC-3') 和 ITS4 (5'-TCCTCCGCTTATTGATATGC-3')。PCR 反应体系为 25.0 μ L 体系: 2 \times PCR Mix 试剂 12.0 μ L、引物各 2.0 μ L、dd H₂O 9.0 μ L、模板 1.0 μ L。PCR 扩增反应条件: 95 $^{\circ}$ C 4 min; 94 $^{\circ}$ C 1 min、55 $^{\circ}$ C 40 s、72 $^{\circ}$ C 1 min, 35 个循环; 72 $^{\circ}$ C 10 min。凝胶电泳检测扩增产物, 并送至上海生工生物工程有限公司测序。将所测序列提交 GenBank 数据库中并进行 Blast 检索,

下载同源性较高的数据, 用 ClustalW 对所得序列进行人工校正及比对分析, 利用 Mega5.02 生成 Fasta 格式的文件, 按照邻接法聚类(选择 bootstrap 检验值 $\geq 50\%$, 1000 次重复) 构建 N-J 系统发育树。

1.4 产酶菌株的筛选

采用透明圈法, 将纯化后的单菌落点接于上述的酶筛选培养基, 每个菌落在平板上点接 3 个重复, 28 $^{\circ}$ C 恒温培养 3-7 d。待菌株生长良好时, 测量不同菌株的透明圈直径和菌落直径, 以透明圈直径与菌落直径的差值 (D) 表示酶活大小, D 值越大, 表明酶活越强。

2 结果和分析

2.1 耐盐真菌的分离

利用 7 种分离培养基从南海局部海域沉积物样品中得到 1689 株海洋真菌, 共计 41 个形态种。其中优势菌株是 95a 和 87b, 其分离频率分别为 13.36% 和 10.82%, 而分离频率最低的菌株为 9m、40n、77a 和 85b, 分离频率均为 0.36%。11 个海洋沉积物样品分离得到真菌的比例依次是 15.45% (261 株)、7.46% (126 株)、5.57% (94 株)、15.87% (268 株)、13.68% (231 株)、5.39% (91 株)、1.60% (27 株)、14.33% (242 株)、5.74% (97 株)、4.14% (70 株)、10.78% (182 株)。其中 4 号样分离出的真菌数最多, 7 号样分离出的真菌最少。41 个形态种中部分耐盐真菌较为特殊, 见图 1。

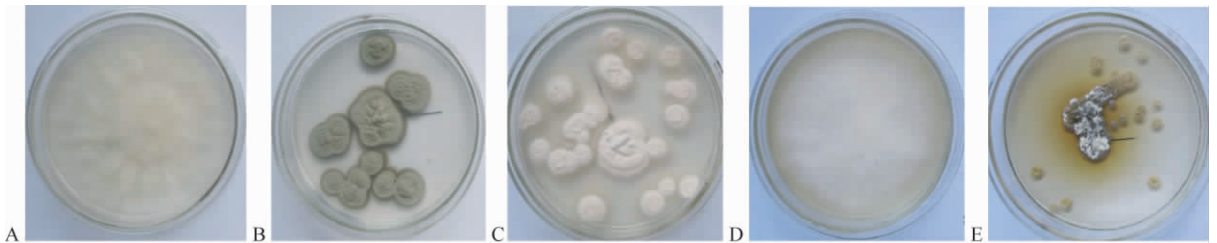


图 1. 部分培养 7 d 耐盐真菌平板

Figure 1. Some purified salt-tolerant fungi from the South China Sea. A: front view of 7m on YPD plate; B: front view of 8m on YPD plate; C: front view of 25m on YPD plate; D: front view of 71a on YPD plate; E: front view of 86b on YPD plate.

2.2 耐盐真菌物种多样性研究

将已纯化的 41 种耐盐真菌各 1 株接种在 YPD 培养基上, 观察菌株的形态特征; 并进行 ITS 序列分析, 初步确定真菌的分类地位(表 2)。结果表明 41

株真菌中散囊菌纲 (Eurotiomycetes) 24 株, 约占 58.50%; 粪壳菌纲 (Sordariomycetes) 8 株, 约占 19.50%; 座囊菌纲 (Dothideomycetes) 3 株, 约占 7.32%; 黑粉菌纲 (Ustilaginomycetes) 和半子囊菌纲

(Hemiascomycetes) 均占 4.88% (2 株); 子囊菌纲 (Ascomycetes) 和伞菌纲 (Agaricomycetes) 均占 2.44% (1 株), 这两个类群相对最少。这些菌株归类于 15 个属, 其中, 曲霉属 (*Aspergillus*) 和青霉属 (*Penicillium*) 是优势菌群, 分别占 39.00% 和 12.20%; 枝顶孢属 (*Acronium*) 约占 7.32%;

Myrmecridium、枝孢属 (*Cladosporium*)、毛壳菌属 (*Chaetomium*)、*Pseudozyma* 和酵母属 (*Saccharomyces*) 均占 4.88%; 散囊菌属 (*Eurotium*)、拟青霉属 (*Paecilomyces*) 和 *Dichotomomyces*、*Acrodontium*、链格孢属 (*Alternaria*)、烟管菌属 (*Bjerkandera*)、赤霉菌属 (*Gibberella*) 均占 2.44%。

表 2. 41 株耐盐真菌的形态特征及分类学地位

Table 2. Morphological features and taxonomic status of 41 salt-tolerant fungi

No.	frequency / %	GenBank accession No.	identity / %	strain (BLAST)	characteristic	taxon
6m	1.10	KC834786	99	<i>Myrmecridium schulzeri</i>	Colonies smaller, flat, pale orange, short filaments. Hyphae separated, not branched; free of spores.	Sordariomycetes
7m	1.41	KC834787	99	<i>Dichotomomyces cejpii</i>	Colonies pale yellow, texture loose, white point appear. Upright and have branches; conidia round.	Eurotiomycetes
8m	0.55	KC834788	96	<i>Acrodontium</i> sp.	Colonies grey, powdery, reverse black. No isolation, slender, branched; conidia string, ovate.	Ascomycetes
9m	0.36	KC834789	100	<i>Cladosporium cladosporioides</i>	Colonies blackish green, reverse black. Separated, fewer branches; short spore chain, ovate.	Dothideomycetes
11m	1.41	KC834790	99	<i>Bjerkandera adusta</i>	White powdery, cream color edges. Transparent hyphae, less branches; spores long elliptic.	Agaricomycetes
12m	3.70	KC834791	99	<i>Paecilomyces variotii</i>	Colonies initially faint yellow, gradients for yellowish brown, powdery, hyphae dense. Branching; conidia string, oval.	Eurotiomycetes
13m	0.38	KC834792	99	<i>Aspergillus sclerotiorum</i>	Colonies initially white, gradients for yellow. Separated, branches, conidial heads spherical; conidia string, spherical.	Eurotiomycetes
15m	0.41	KC834793	100	<i>Penicillium chrysogenum</i>	Colonies initially white, gradients for cyan, cyan powdery. Upright, conidial heads line up brush; conidia string, spherical.	Eurotiomycetes
16n	4.23	KC834794	99	<i>Aspergillus versicolor</i>	Colonies initially bluish yellow, gradients for gray blue. Transparent conidial heads loose radial, spherical; conidia string, spherical.	Eurotiomycetes
17m	0.41	KC834795	100	<i>Penicillium chrysogenum</i>	Colonies gray blue, reverse slightly yellow. Less branches, conidial heads line up brush; conidia string, spherical.	Eurotiomycetes
18m	1.03	KC834796	99	<i>Eurotium herbariorum</i>	Colonies yellow and green line to line, central to gray. Hyphae slender, branched, ascoma for cleistothecium, globose; conidium spherical.	Eurotiomycetes
19n	1.10	KC834797	100	<i>Aspergillus flavus</i>	Colonies initially white, gradients for yellow-green. No branching, conidial heads loose radial, spherical; conidia spherical.	Eurotiomycetes
21m	1.10	KC834798	100	<i>Aspergillus niger</i>	Colonies initially white, filiform, gradients for black-brown. No branching, conidial heads spherical; conidia string, spherical, brown.	Eurotiomycetes

续表 2

No.	frequency / %	GenBank accession No.	identity / %	strain (BLAST)	characteristic	taxon
22m	1. 41	KC834799	99	<i>Aspergillus carneus</i>	Colonies dark grey , edges irregular. No branching , conidial heads like a brush; conidia string , spherical.	Eurotiomycetes
25m	0. 76	KC834800	95	<i>Acremonium</i> sp.	Colonies pink-white , reverse dark yellow. Hyphae slender , conidia ovate.	Sordariomycetes
30m	0. 41	KC834801	99	<i>Aspergillus versicolor</i>	Colonies initially white , gradients for yellow-green , central flocculent , reverse yellow. No branching , conidial heads spherical , loose radial; conidia string.	Eurotiomycetes
31m	3. 70	KC834802	100	<i>Alternaria alternata</i>	Colonies initially white villose , gradients for gray; reverse is grayish-brown. No isolation , bending , branches; free of spores.	Dothideomycetes
34m	0. 83	KC834803	99	<i>Aspergillus sydowii</i>	Colonies darkcyan. No branching , conidial heads spherical; conidia string , spherical.	Eurotiomycetes
35n	0. 37	KC834804	100	<i>Aspergillus tubingensis</i>	Colonies initially white , gradients for black-brown , reverse black. No branching , conidial heads spherical; conidia spherical , brown.	Eurotiomycetes
36m	1. 41	KC834805	100	<i>Aspergillus versicolor</i>	Colonies cyan , radial edges. No branching , conidial heads spherical; conidia string , spherical.	Eurotiomycetes
38m	1. 06	KC834806	100	<i>Aspergillus versicolor</i>	Colonies darkcyan. No branching , conidial heads spherical; conidia string , spherical.	Eurotiomycetes
39m	0. 37	KC834807	100	<i>Aspergillus fumigatus</i>	Colonies bluish green , filiform. No branching , conidial heads spherical , loose radial; conidia string , spherical.	Eurotiomycetes
40n	0. 36	KC834808	100	<i>Penicillium commune</i>	Colonies darkcyan , radial edges. Less branches; conidia string , spherical.	Eurotiomycetes
41m	0. 79	KC834809	100	<i>Penicillium citrinum</i>	Colonies darkcyan. No branching , conidial heads line up brush; conidia string , spherical.	Eurotiomycetes
43m	1. 41	KC834810	99	<i>Aspergillus penicillioides</i>	Colonies darkcyan , greenish-white edges. No branching , conidial heads spherical , loose radial; conidia string , spherical.	Eurotiomycetes
45m	3. 72	KC834811	100	<i>Penicillium chrysogenum</i>	Colonies darkcyan , edges radial; Less branches , conidial heads line up brush; conidia spherical.	Eurotiomycetes
71a	2. 20	KC834812	100	<i>Gibberella intermedia</i>	Colonies white villous , densely , yellow small droplets; reverse dark white. Separated , bent , more branches; conidia ovate.	Sordariomycetes
72a	1. 41	KC834813	99	<i>Aspergillus versicolor</i>	Colonies ivory white , fleshy , have fold. No isolation , curved; conidial heads shaped branches , conidia string , spherical.	Eurotiomycetes
77a	0. 36	KC834814	99	<i>Aspergillus versicolor</i>	Colonies white powdery. Hyphae slender , branched; ascoma for cleistothecium , oval.	Eurotiomycetes
78a	0. 37	KC834815	99	<i>Myrmecridium schulzeri</i>	Colonies ivory white , small , short filaments , edge neat. Hyphae slender , isolation , bending.	Sordariomycetes

续表 2

No.	frequency / %	GenBank accession No.	identity / %	strain (BLAST)	characteristic	taxon
82b	0.79	KC834816	99	<i>Chaetomium globosum</i>	Colonies white villous, densely, having protuberance. Hyphae slender, isolation, curved; ascoma nearly spherical, organized by textura intricata.	Sordariomycetes
84b	10.33	KC834817	99	<i>Pseudozyma antarctica</i>	Colonies initially pink white, gradients for pale pink, staggered linear, texture dense. Less branches; conidia ovoid.	Ustilaginomycetes
85b	0.36	KC834818	99	<i>Acremonium strictum</i>	Colonies pink-white, reddish, fleshy, glossy. Separated, less branches; conidia ovate.	Sordariomycetes
86b	3.70	KC834819	100	<i>Aspergillus sclerotiorum</i>	Colonies initially light green, gradients for pale chartreuse, white patches, produce black pigment. Hyphae slender, curved, having branches.	Eurotiomycetes
87b	10.82	KC834820	99	<i>Aspergillus sclerotiorum</i>	Colonies pink, smooth, texture sticky. Conidia rounded, overlap together.	Eurotiomycetes
89b	0.79	KC834821	100	<i>Pseudozyma antarctica</i>	Colonies pink, threadiness. Conidiophore bent, less branches; conidia long rods.	Ustilaginomycetes
90a	0.41	KC834822	100	<i>Chaetomium globosum</i>	Colonies central is white, density, outward is relatively loose. Separated, curved; ascoma nearly spherical, organized by textura intricata.	Sordariomycetes
95a	13.36	KC834823	95	<i>Acremonium</i> sp.	Colonies snow-white, texture dense. Separated, bending, branches; conidia ovate.	Sordariomycetes
96c	9.54	KC834824	100	<i>Saccharomyces cerevisiae</i>	Colonies orange, coarse, more sticky, opaque, small colonies. Cells are short rod.	Hemiascomycetes
98c	9.54	KC834825	100	<i>Saccharomyces cerevisiae</i>	Colonies orange, smooth, more sticky, opaque. Cell ovoid, cluster-type arrangement.	Hemiascomycetes
100a	0.37	KC834826	99	<i>Cladosporium tenuissimum</i>	Colonies initially green, white edges, gradients for ash black, villous. Separated; conidia ovate, torose spore chains.	Dothideomycetes

Isolation frequency = The number of strain / the total number in this sample.

南海局部沉积物耐盐真菌 ITS 序列同 NCBI 数据库中相关 ITS 序列相似性为 95% - 100% 之间。N-J 系统发育研究表明 (图 2), Bootstrap 值在 51% - 100% 之间, 归于 15 个不同的属, 如 *Aspergillus*、*Penicillium*、*Acremonium*、*Myrmecridium*、*Cladosporium* 和 *Chaetomium*。此外, 菌株 25m、95a 和 8m 分别同 *Acremonium implicatum* (AF368810)、*A. implicatum* (AF368810) 和 *Acrodontium crateriforme* (AY843112) 的序列相似性为 95% - 96%, 可能是潜在的新物种, 需进一步研究确定。

2.3 菌株产酶活性研究

41 株已测序的耐盐真菌菌株的产酶活性研究表明 (表 3) 8 株真菌产纤维素酶, 9 株真菌产淀粉

酶, 5 株真菌产复合酶 (包括蛋白酶、淀粉酶及木聚糖酶活性), 16 株真菌产蛋白酶, 3 株真菌产脂肪酶, 未发现产壳聚糖酶的菌株; 其中有 7 株真菌同时产 3 种酶, 4 株真菌同时产两种酶。产酶菌株共有 23 株, 分布在 10 个属, 其中曲霉属 (*Aspergillus*) 9 株, 青霉属 (*Penicillium*) 4 株, 枝顶孢属 (*Acremonium*) 3 株, 枝孢属 (*Cladosporium*)、*Myrmecridium*、链格孢属 (*Alternaria*)、*Pseudozyma*、*Dichotomomyces*、烟管菌属 (*Bjerkandera*) 和 *Acrodontium* 均只有 1 株。产酶活性大小研究表明, *Acrodontium* sp. 8m 和 *Aspergillus* sp. 86b 产复合酶的活性相对较高, *Penicillium* sp. 41m 产蛋白酶的活性相对较高。

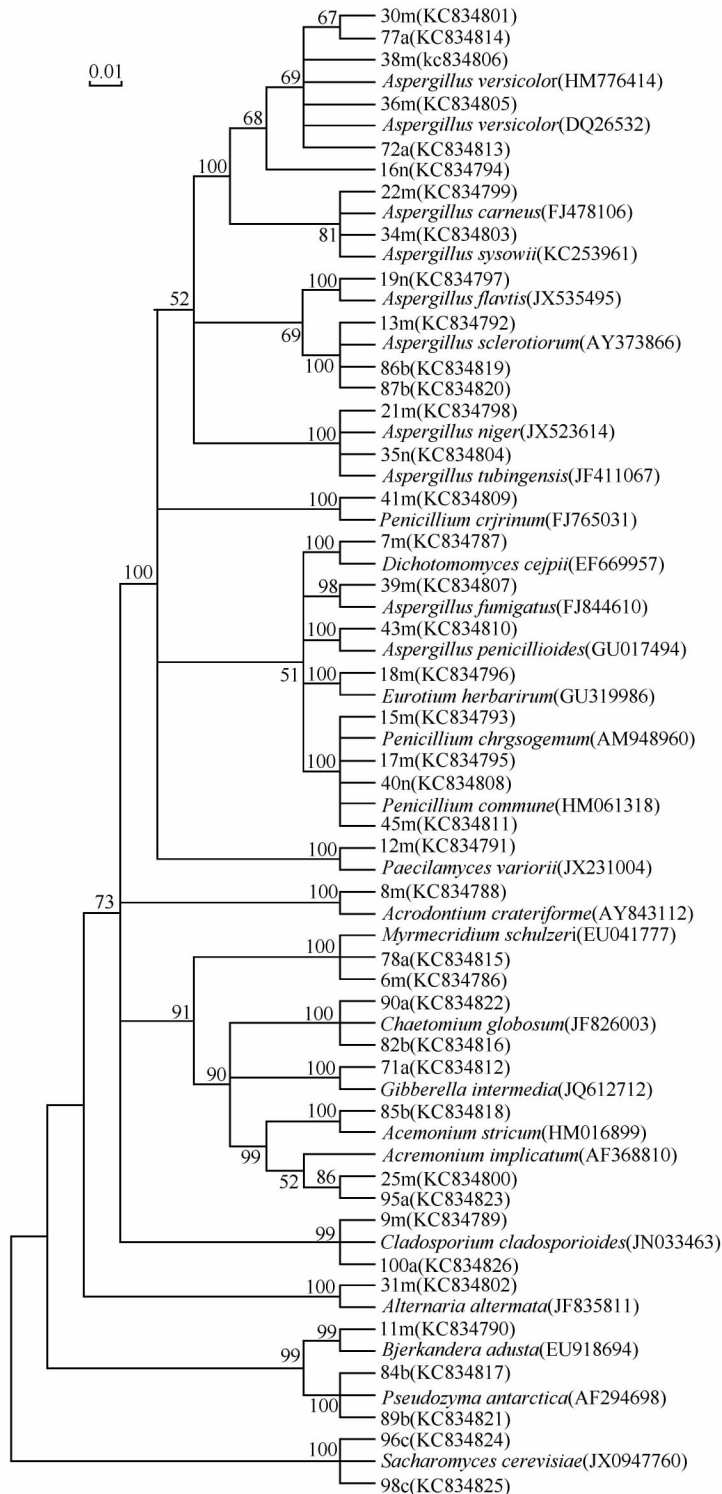


图 2. 基于 rDNA ITS 基因构建的耐盐真菌系统发育树

Figure 2. Phylogenetic tree of halotolerant fungi based on rDNA ITS gene sequences by using neighbour-joining method, numbers at branching points refer to bootstrap values (1000 resamplings; only values above 50% were shown), 0.01 was sequence divergence. Numbers in parentheses represent the sequences accession number in GenBank.

表 3. 产透明圈菌株的 D 值

Table 3. D values of transparent circles by enzyme producing strains

No.	cellulase	amylase	compound enzyme	protease	lipase	chitonsanase
6m	++	-	-	+++	++	-
7m	-	-	-	+++	-	-
8m	-	-	++++	-	-	-
9m	-	+	-	+++	++	-
11m	-	-	+++	++	-	-
15m	++	-	-	-	-	-
25m	+++	-	+++	++	-	-
30m	-	-	-	++	-	-
31m	-	++	-	-	-	-
35n	++	-	-	-	+++	-
36m	-	-	-	++	-	-
38m	-	++	+++	++	-	-
39m	-	-	-	+++	-	-
40n	-	++	-	-	-	-
41m	++	++	-	++++	-	-
43m	-	++	-	++++	-	-
45m	++	-	-	-	-	-
72a	-	-	-	+++	-	-
77a	-	-	-	++	-	-
84b	-	++	-	-	-	-
85b	+++	++	-	++	-	-
86b	-	-	++++	++	-	-
95a	+++	++	-	+++	-	-

“++++”: 20–25 mm “+++”: 15–20 mm “++”: 10–15 mm “+”: 5–10 mm “”: 0–5 mm “-”: No transparent circle.

3 讨论

海洋沉积物是一个具有丰富物种多样性的巨大宝库,其中的耐盐真菌作为海洋微生物的一大类群,因其生境的特殊性而具有巨大的开发潜力。本文通过平板涂布法从南海局部采集的 11 份海洋沉积物中分离得到 1689 株海洋真菌,共 41 个形态种。经形态学研究和 ITS 序列分析分析表明,南海局部海洋沉积物中的耐盐真菌物种多样性较为丰富,归类于 15 个属,其中曲霉属(*Aspergillus*)和青霉属(*Penicillium*)为优势菌群,分别占 39.00% 和 12.20%。黄菁菁等^[24]利用平板涂布法从福建厦门、晋江及诏安沿海等 4 种生境采集到的样品中分离得到的耐(嗜)盐真菌以青霉和曲霉为主,分别占全部活性菌株的 46.6% 和 25.5%;孙开明^[25]利用平板涂布法从黄海山东沿岸的沉积物中分离出的真菌青霉属最为广泛,均与本研究结果不同。徐新霞等^[7]利用平板涂布法对采集的深海

沉积物样品进行了真菌分离,其中曲霉属和青霉属最多,占 50%,与本研究结果基本相同。此差异性的出现可能与采样地点的生长环境有关。此外,本研究还发现海洋真菌菌株的多样性与取样深度无明显的相关性,可能与取样点的可利用 C 源、N 源,温度、pH 值等其他生物因素的影响相关。

本研究共获得 13 个稀有属,如 *Chaetomium*、*Eurotium*、*Myrmecridium* 和 *Acremonium* 等。通过比较分析发现,在以前他人的研究中也有一部分稀有属被报道,如王松等^[26]从青岛近海的红藻多管藻中获得 *Chaetomium globosum*; Bass 等^[27]从 11 份世界各地的深海样品中发现 *Eurotium herbariorum* 和 *Saccharomyces cerevisiae*;冯春辉^[28]采用稀释平板法和土壤平板法从长江口、南黄海、北黄海三大海域采集的 48 份沉积物样品中分离到 *Acremonium strictum* 和 *Alternaria alternata*; Zhang 等^[13, 29]从先后从南海柳珊瑚和南海深海沉积物中分离得到 *Paecilomyces variotii*、*Myrmecridium schulzeri*、*Gibberella intermedia*、

Acremonium sp.、*Cladosporium cladosporioides* 和 *C. tenuissimum*。而有些属在前人的研究中被报道,本研究中却未发现,如 Zhang 等^[13]发现的 *Arthrinium*、*Catenulostroma*、*Rhodotorula*、*Aureobasidium*、*Engyodontium* 和 *Phaeosphaeriopsis*。但有些稀有种属在海洋环境中未见报道,如菌株 *Acrodontium* sp. 8m、*Bjerkandera adusta* 11m、*Pseudozyma antarctica* 84b 和 89b,其中菌株 8m 的序列相似性为 96%,可能为潜在的新种。这些分析表明南海局部海洋沉积物中具有重要、新颖的海洋真菌资源,值得进一步研究。

海洋真菌产生的酶类,往往具有其它陆源真菌酶所不具有的特性,如耐盐、耐压、耐酸碱、耐高低温等特性。目前,尚未有其他研究发现产多种酶活性的海洋真菌;而本研究发现,有 7 株真菌同时产 3 种酶,4 株真菌同时产两种酶。产酶菌株共有 23 株,分布在 10 个属,其中曲霉属和青霉属最多,占产酶菌株的 56.52%。迄今为止,人们对海洋真菌的产酶特性研究仍然停留在真菌产某种酶的阶段,发现的产酶菌株大多数为青霉属或曲霉属^[18,22],这与本研究的结果一致。本研究还发现 *Acrodontium* sp. 8m 和 *Aspergillus* sp. 86b 产复合酶的活性相对较高,*Penicillium* sp. 41m 产蛋白酶的活性相对较高。结合产酶和系统发育分析可以看出同一属的不同种,其产酶特性存在很大的差别,比如菌株 13m 和 38m 均归为曲霉属,菌株 13m 不产实验中的 6 种酶,而菌株 38m 却产 3 种酶。关于产酶条件的进一步优化正在研究中,以期能够寻找到产酶量较大且具有较高酶活性的菌株。

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Fungal diversity and enzyme activities in marine sediments in the South China Sea

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Abstract: [Objective] We studied species diversity and enzyme activities of fungi from 11 marine sediment samples in the South China Sea. [Methods] Salt-tolerant fungi were isolated by a dilution-plate method, and their diversity was studied based on fungal morphology and rDNA ITS (Internal Transcribed Spacer) sequences. Enzyme activities were screened by six special selective media. [Results] A total of 1689 strains of salt-tolerant fungi were isolated. Morphology and ITS sequence analysis identified these fungi to 41 species of 15 described genera, of which *Aspergillus* sp. and *Penicillium* sp. were the dominant populations. Studies on enzyme activities of 41 sequenced strains showed that 8 strains produce cellulase, 9 strains produce amylase, 5 strains produce compound enzyme, 16 strains produce protease, 3 strains produce lipase and no strain produce chitonsanase. *Acrodontium* sp. 8m and *Aspergillus* sp. 86b produced the most multiple enzymes, while *Penicillium* sp. 41m produced comparatively higher protease. [Conclusion] There were abundant salt-tolerant fungi from marine sediment samples in the South China Sea, and more strains had enzyme activities.

Keywords: Marine sediments, salt-tolerant fungi, species diversity, enzyme activities

(本文责编: 张晓丽)

Supported by the Key Project of China National Programs for Fundamental Research and Development (2010CB833801), by the National Natural Science Foundation of China (31100017) and by the Youth Nova Project of Science and Technology in Shaanxi Province (2013KJXX-76)

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Received: 14 August 2013 / Revised: 30 November 2013