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坛紫菜养殖周期中的藻际微生物多样性

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摘要:【目的】坛紫菜是我国江浙海区栽培地主要经济藻类。观察紫菜养殖过程中藻际微生物的群落特点及变化,研究藻际环境中的微生物因素在紫菜栽培中的作用,为保证紫菜健康生长及病害防治提供理论与实验基础。【方法】采用传统纯培方法和 PCR-DGGE 技术分离归类坛紫菜养殖周期中的藻际微生物,并利用 16S rDNA(细菌)和 18S rDNA(真菌)序列测定及在线 BLAST 比对鉴定到属,比较分析不同生长阶段、不同养殖海区及养殖过程的坛紫菜藻际微生物的多样性特点。【结果】在坛紫菜养殖过程中总共分离到 467 株细菌,共 41 个属。分类结果显示藻际细菌归属于变形菌门(Alphaproteobacteria 和 Gammaproteobacteria)、放线菌门(Actinobacteria)、厚壁菌门(Firmicutes)和拟杆菌门(Bacteroidetes),优势菌群为 α-变形菌纲和 γ-变形菌纲。分离到 55 株真菌,共 15 个属。分类结果显示绝大多数真菌归属于子囊菌门(Ascomycota),仅 1 株归属于担子菌门(Basidiomycota)伞菌纲(Agaricomycetes)。细菌多样性大于真菌。坛紫菜藻际细菌有 19 个特异菌属,对照海水细菌有 13 个特异菌属;从丝状体中分离到大部分真菌和放线菌,坛紫菜养殖丝状体和不同叶状体养殖阶段的藻际微生物类别差异明显。在分离的坛紫菜藻际微生物中发现了与引起细菌性红烂病的海科贝特菌(*Cobetia marina*)、引起白斑病的紫菜茎点菌(*Phoma porphyrae*)高度相似的菌株,以及与典型的腐霉如镰孢霉菌(*Fusarium* sp.)和曲霉(*Aspergillus* sp.)高度相似的菌株。【结论】坛紫菜养殖过程中藻际微生物的多样性受到紫菜生长形态、养殖时间及养殖环境等因素的影响。在藻际微生物中发现与紫菜致病菌高度相似的微生物,作为潜在致病微生物应得到重视。

关键词:坛紫菜,细菌,真菌,多样性,养殖周期
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坛紫菜(*Pyropia haitanensis*)[1]是中国浙江和福建海区的地方种,也是该地区主要的紫菜栽培品种,每年创造数 10 亿产值。坛紫菜养殖周期分为海区养殖(叶状体阶段)和苗场育苗(丝状体阶段)两大阶段。坛紫菜采用分期采割,叶长 15 cm – 20 cm 即可采收 1 次,从秋后开始可持续到翌年 3 – 5 月,其中初期从 9 月中旬到 11 月下旬,中期从 12 月上旬到翌年 2 月下旬,后期从 3 月上旬到 4 月上旬。采苗季节在 4 月初,进行采果孢子或者接种自由丝状体,然后平育或吊育,直到 9 月初再采壳孢子苗到网帘并下海培育[2]。随着紫菜人工养殖面积的不断增加,养殖环境的恶化,养殖管理的人为疏漏及养殖密度不断增加,

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每年都有不同程度的养殖病害发生。除本身的种质退化、生理性不适、环境等因素外，病原菌感染也是引起紫菜病害的重要原因。例如坛紫菜叶状体细菌性红烂病由海科贝特菌(*Cobetia marina*)引起[3-4]；紫菜叶状体绿斑病的致病菌多数为假单胞菌属(*Pseudomonas*)[5-6]，其中条斑紫菜的主要病原菌为柠檬假交替单胞菌(*Pseudoalteromonas citrea*)[7]；紫菜叶状体赤腐病主要由紫菜腐霉菌(*Pythium porphyrae*)引起[8]；紫菜叶状体壶疫病由拟油壶菌属(*Olpidiopsis* spp.)引起[9-10]；条斑紫菜丝状体黄斑病的致病菌可能为河豚毒素交替假单胞菌(*Pseudoalteromonas tetraodonis*)[11]；条斑紫菜丝状体白斑病可能由紫菜茎点菌(*Phoma porphyrae*)引起[12]。

1972年Bell和Mitchell[13]提出了“藻际微环境”这一概念，为揭示藻类健康生长提供了新的视点。藻类不断向周围环境释放氨基酸及其他代谢产物，为共生、共栖或寄生的藻际微生物提供营养，并进行筛选[14-15]；同时藻际微生物也为藻类提供生长调节因子或营养素[16]。藻类和微生物相互选择、相互依存甚至相互拮抗，处于动态平衡之中，各取所需，协同发展；一旦环境变化或物种入侵导致正常的藻菌相互体系受到破坏，微生物群落失衡，则有可能造成藻类病害。陈国耀等[17]连续2年对条斑紫菜叶状体附生菌及病原菌做了研究，发现黄色和橘红色菌落占优势，健康养殖紫菜和周围海水的细菌数量保持稳定，而病烂紫菜上有较多的降解琼脂细菌。杨锐等[18]研究了4地条斑紫菜叶状体和丝状体的细菌遗传多样性，发现在健康样品中存在的假交替单孢菌并未从病烂样品中分离到，提示假交替单孢菌可能与紫菜健康生长有密切关系。通过观察紫菜养殖过程中藻际微生物的群落特点及变化，或追踪潜在的致病菌，可能为紫菜健康栽培及病害防治提供有力的微生态依据。

本文在坛紫菜养殖的2个阶段4个时间点采样，研究坛紫菜养殖过程中的藻际微生物多样性，有助于了解坛紫菜养殖过程中的微生物组成变化，为优化紫菜养殖环境和预测防治紫菜病害提供理论依据，并为后续藻菌互作研究提供关键菌株。

1 材料和方法

1.1 主要试剂和仪器

Zobell 2216E海水培养基(酵母粉1 g，蛋白胨5 g，柠檬酸铁0.1 g，琼脂15–20 g，陈海水定容至1000 mL，pH7.6–7.8)，马铃薯海水(PDA)培养基(马铃薯200 g，蔗糖20 g，琼脂15–20 g，陈海水定容至1000 mL，pH自然)，PCR反应试剂(TaKaRa)，变性梯度凝胶电泳及银染试剂(上海捷瑞生物工程有限公司)，Ezup柱式基因组DNA抽提试剂盒(生工生物工程上海有限公司)，Biospin真菌基因组DNA抽提试剂盒(杭州博日科技有限公司)，显微镜(Olympus)，PCR扩增仪(Eppendoff)，电泳仪及紫外凝胶成像系统(BIO-RAD)，变性梯度凝胶电泳系统(BIO-RAD)等。

1.2 采样与微生物的分离纯化

1.2.1 采样：本实验室从浙江温州紫菜养殖区采集了健康坛紫菜(*P. haitanensis*)丝状体阶段(2010-04和2010-09)和叶状体阶段(2010-11和2011-03)共4个时段的贝壳丝状体、紫菜叶状体及其环境海水样品，测定海区温度、盐度和pH值(表1)，现场处理得到海水样品(SW)、贝壳研磨滤液(C)、紫菜振荡悬液(S)和紫菜研磨悬液(G)，方法参考文献[18]。

表1. 采样时间地点、环境因子和分离微生物数

Table 1. Sampling time, location, environmental factors and isolates number

Sampling No.	Time	Location	Environmental factors			Bacterial isolates	Fungal isolates
			Temperature/℃	pH	Salinity/‰	number	number
1	2010.04	Cangnan, Wenzhou(温州苍南)	15.6	7.54	17	178	33
2	2010.09	Cangnan, Wenzhou(温州苍南)	28.2	7.34	31	119	14
3	2010.11	Pingyang, Wenzhou(温州平阳)	16.7	7.98	27	102	6
4	2011.03	Pingyang, Wenzhou(温州平阳)	11.0	7.96	26	68	2
Total						467	55

1.2.2 分离微生物：将上述4种悬液分别涂布于Zobell 2216E海水培养基和马铃薯海水(PDA)培养基30℃倒置培养。细菌培养2–3 d，真菌培养5–7 d，按菌落形态特征挑取不同单菌落，分区划线

2–3次进行分离纯化,革兰氏染色并显微镜镜检后接种于相应斜面培养基4℃保存和部分细菌甘油管(10% –20%) –20℃保种。分离到的菌株统一编号,B代表细菌,F代表真菌,W 1-4代表温州代表采样的时间顺序,Ph代表来自于坛紫菜,如BW1SW1表示分离自第1次温州采样的海水细菌,FW2PhS1表示分离自第2次温州采样的坛紫菜振荡处理真菌。

1.3 细菌16S rDNA和真菌18S rDNA基因序列分析

1.3.1 基因组DNA的抽提:取30℃培养18–24 h的细菌1–3 mL,用Ezup柱式基因组DNA抽提试剂盒提取基因组DNA。取30℃培养2–3 d的真菌(出现菌丝球)1–3 mL,充分研磨后用Biospin真菌基因组DNA抽提试剂盒提取基因组DNA。取5 μL DNA样品进行1% 含溴化乙锭琼脂糖凝胶电泳(100 V,40 min),在紫外凝胶成像系统下拍照检测。提取的基因组DNA保存于–20℃备用。

1.3.2 PCR-变性梯度凝胶电泳(PCR-DGGE)分析:PCR反应条件:95℃ 8 min;95℃ 30 s,58℃ 45 s,72℃ 2 min,35个循环;72℃ 10 min。取5μL PCR产物进行1% 含溴化乙锭琼脂糖凝胶电泳(100 V,40 min),在紫外凝胶成像系统下拍照检测并保存于–20℃备用。采用细菌通用引物GC + 338F和518R(表2)[19]和真菌通用引物GC + FR1和FF390(表2)[20]分别对16S rDNA的V3区和18S rDNA可变区进行扩增,用于变性梯度凝胶电泳(DGGE)分析。变性梯度凝胶电泳采用8%聚丙烯酰胺凝胶,变性剂尿素和甲酰胺的变性梯度为40% –55%[21]。点样量为8 μL PCR产物和8 μL 2×上样缓冲液,电压180 V,温度55℃,电泳6 h后银染拍照比对条带。

表2. 引物序列

Table 2. Primer sequences of Polymerase Chain Reaction (PCR)

Target sequence	Sequence length/bp	Primer sequences (5′→3′)
Variable region of 16S rDNA	About 180	GC-Clamp + 338F: CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTA CGGGAGGCAGCAG
		518R: ATTACCGCGGCTGCTGG
16S rDNA	About 1500	27F: AGAGTTTGATCCTGGCTCAG
		1492R: AAGGAGGTGATCCAGCCGCA
Variable region of 18S rDNA	About 400	GC-Clamp + FR1: CCCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGCCGAICCATT CAATCGGTAIT
		FF390: CGATAACGAACGAGACCT
18S rDNA	About1800	GeoA2: CCAGTAGTCATATGCTTGTCTC
		Geo11: ACCTTGTTACGACTTTTACTTCC

1.3.3 基因序列测定及同源性分析:采用细菌通用引物27F和1492R(表2)[22]和真菌通用引物GeoA2和Geo11(表2)[23]分别对16S rDNA和18S rDNA进行扩增,PCR产物直接移交上海英俊生物技术有限公司进行测序。PCR反应体系和条件同1.3.2,送测体积为50 μL。得到的序列通过National Center for Biotechnology Information(NCBI)的GenBank数据库进行在线BLAST相似性比对,得到相似度最高的序列,鉴定到属水平。通过Cluster W和MEGA 4软件构建系统发育树。细菌序列登录号为KC012807–KC012879,KC012881–KC012886,KC01288–KC012898,KC012900–KC012906,KC012908–KC012909,真菌序列登录号为JX273049–JX273068。

2 结果

2.1 分离纯化与菌落形态

坛紫菜养殖过程中总共分离纯化得到细菌467株,真菌55株,真菌少于细菌。根据菌落特征和菌体形态将细菌归类为100个表型,淡乳黄短杆状为优势形态细菌类群。其中B1–B5(5类,表3)在4次的采样分离细菌中都有存在,占总分离细菌的24.20%;B6–B18(13类,表4)在3次的采样分离细菌中有存在,占30.41%;B19–B36(18类)在2次的采样分离细菌中有存在,占18.06%;剩余的B37–B100都只在1次的采样分离细菌中有存在,占27.33%。真菌形态归类为23个表型,除F1–F5(5

类,表4)在2次采样分离真菌中有存在,占总分离真菌的36.36%;其余F6-F23类都只在1次的采样分离真菌中存在,占63.64%。除了普遍存在的形态类群外,各个时期都有独特的形态类群,直观地反映了坛紫菜藻际微生物在不同养殖时期的群落动态变化。

表3. 细菌优势形态类群

Table 3. Dominant populations based on bacterial morphology

Bacterial morphological No.	Cell shape	Colony color	Colony shape	Colony size	Colony moisture	Colony edge	Colony surface	Colony transparency	Isolates number
B1	Short rod	Pale yellow fraction	Round	Small	Moist	Complete	Salient, smooth	Opaque	34
B2	Rod	Pale yellow fraction	Round	Small	Moist	Complete	Salient, smooth	Opaque	29
B3	Rod	milky-white	Round	Small	Moist	Complete	Salient, smooth	Opaque	15
B4	Short rod	Pale yellow fraction	Round	Small	Moist	Complete	Salient, smooth	Transparent	18
B5	Rod	Pale yellow fraction	Round	Small	Moist	Complete	Salient, smooth	Transparent	17
B6	Short rod	Pale yellow	Round	Small	dry	Complete	Salient, smooth	Opaque	6
B7	Rod	Pale yellow	Round	Small	Moist	Complete	Salient, smooth	Opaque	7
B8	Short rod	Pale yellow fraction	Round	Large	Moist	Complete	Flat, smooth	Opaque	7
B9	coccus	Pale yellow fraction	Round	Small	Moist	Complete	Salient, smooth	Opaque	13
B10	Short rod	Pink	Round	Small	Moist	Complete	Salient, smooth	Opaque	5
B11	Rod	Orange	Round	Small	Moist	Complete	Salient, smooth	Opaque	6
B12	Short rod	milky-white	Irregular	Large	Few wet	Incomplete	Eminence, smooth	Opaque	4
B13	Short	yellow fraction	Round	Small	Moist	Complete	Salient, smooth	Opaque	14
B14	Short rod	Pale yellow	Round	Small	Moist	Complete	Salient, smooth	Transparent	13
B15	Short rod	Pink	Round	Small	Moist	Complete	Salient, smooth	Transparent	8
B16	Short rod	Orange	Round	Small	Moist	Complete	Salient, smooth	Transparent	14
B17	Rod	Orange	Round	Small	Moist	Complete	Salient, smooth	Transparent	36
B18	Rod	yellow fraction	Round	Small	Moist	Complete	Salient, smooth	Transparent	9
B19-36								89	
B37-B100								123	
Total									467

表4. 真菌优势形态类群

Table 4. Dominant populations based on fungal morphology

Fungal morphological No.	Front color	Back color	Hypha height	Growth density	Surface	Pigment	Isolates number
F1	green, white edge	Yellow	Short	Thick	Powder	No	3
F2	Dark green, white edge	Dark green, white edge	Short	Thick	Down, verrucous	No	6
F3	White	Yellow, white edge	Short	Thick	Down	No	4
F4	Orange	Orange	Short	Thick	Down, radial	No	5
F5	Orange	Orange	Short	Thick	Down, radial	No	2
F6-F23							35
Total							55

2.2 变性梯度凝胶电泳分析

细菌16S rDNA的V3区和真菌18S rDNA可变区PCR产物用于变性梯度凝胶电泳,银染后得到清晰条带进行比对分析(图1为细菌DGGE银染典型条带,图2为真菌DGGE银染典型条带)。理论上DGGE可分辨1个碱基差异的条带,本实验将拥有相同条带的菌株暂归为同一型[24]。细菌分型数按采样先后依次为18、10、57和18,真菌分型数为20,结合测序结果最终确定微生物的种属。

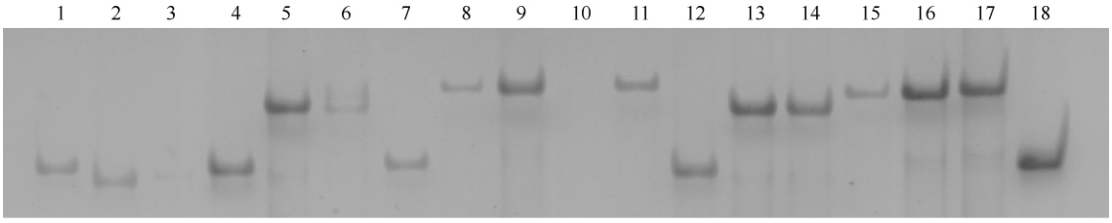

图 1. 细菌 16S rDNA DGGE 典型图

Figure 1. Tipical denaturing gradient gel electrophoresis (DGGE) fingerprint of bacterial 16S rDNA. Lane 1, BW1PhC10; lane 2, BW2PhC21; lane 3, BW2PhC9; lane 4, BW2SW44; lane 5, BW1PhC24-1; lane 6, BW1PhC24-3; lane 7, BW2SW30; lane 8, BW2SW45; lane 9, BW1SW6; lane 10, BW1SW39-4, no band; lane 11, BW2SW10; lane 12, BW1PhC46-1; lane 13, BW1SW28-1; lane 14, BW1SW28-2; lane 15, BW2PhC43; lane 16, BW1SW21; lane 17, BW1SW20; lane 18, BW1SW38-1.

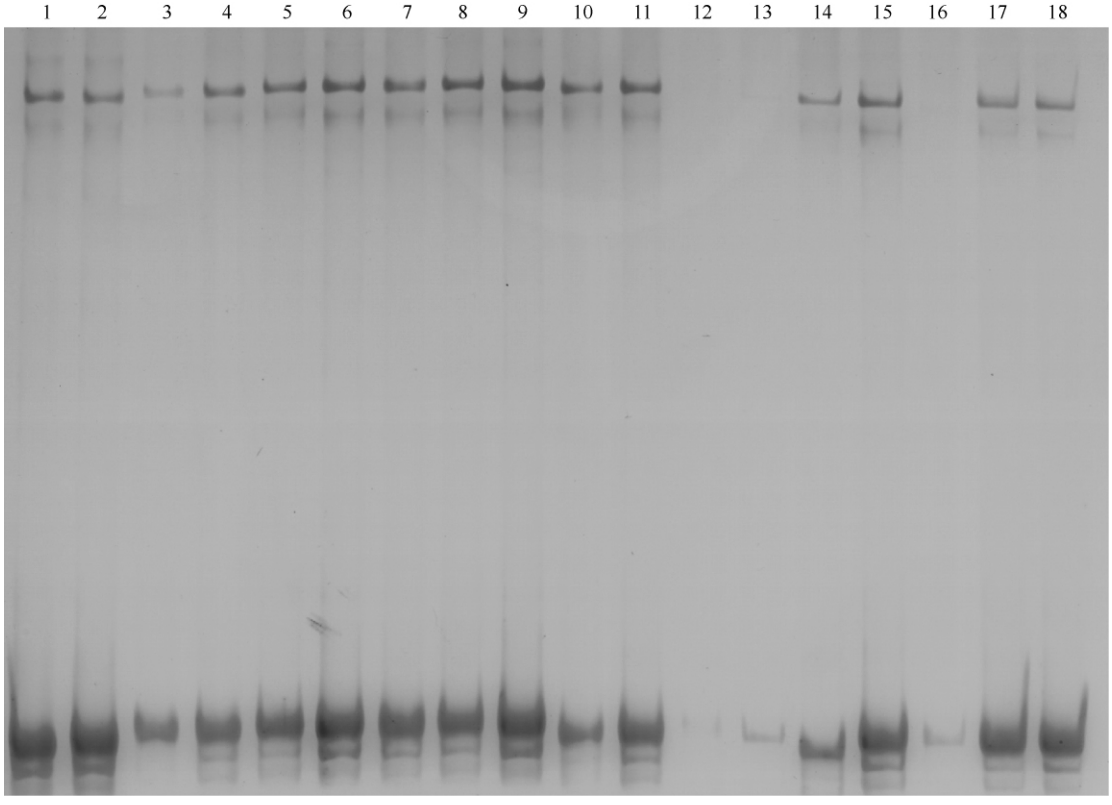

图 2. 真菌 18S rDNA DGGE 典型图

Figure 2. Tipical denaturing gradient gel electrophoresis (DGGE) fingerprint of fungal 18S rDNA. Lane 1, FW3PhS1; lane 2, FW4SW1; lane 3, FW1PhC4-1; lane 4, FW2SW3; lane 5, FW2SW2; lane 6, FW4PhS1; lane 7, BFW3PhG2; lane 8, FW3PhG3; lane 9, FW3SW1; lane 10, FW3PhG1; lane 11, FW2SW1; lane 12, FW1PhC5-1; lane 13, FW1PhC5-2; lane 14, FW2SW9; lane 15, FW2PhG1; lane 16, FW1SW4; lane 17, FW1SW1; lane 18, FW1SW2.

2.3 微生物的分子鉴定及群落特征

综合 DGGE 分析和测序比对结果，总共得到 99 株基因型不同的细菌，分属 41 个属(表 5)，20 株基因型不同的真菌，分属 15 个属(表 6)。从丝状体分离到的细菌 *Cobetia* spp. 与前文提到的坛紫菜叶状体红烂病致病菌归属相同，真菌 *Phoma* spp. 与条斑紫菜丝状体白斑病致病菌归属相同。对细菌的分布差异进行比较，发现(1)芽胞杆菌属(*Bacillus*)和副球菌属(*Paracoccus*)普遍存在于坛紫菜养殖的各个阶段中；2010-04 和 2011-03 的 2 个时期都分离到冷杆菌属(*Psychrobacter*)、节杆菌属(*Arthrobacter*)和喜盐芽胞杆菌属(*Halobacillus*)，这可能与当时初春海水温度较低有关。(2)坛紫菜共附生细菌和对照海水细菌存在明显差异：坛紫菜共附生细菌分布于 28

个属，多于海水细菌分布的 20 个属。*Sulfitobacter*、*Phaeobacter*、*Roseivivax*、*Loktanella*、副球菌属（*Paracoccus*）、赤杆菌属（*Erythrobacter*）、*Ahrensia*、*Robiginitomaculum*、*Sphingopyxis*、芽单胞菌属（*Blastomonas*）、寡养单胞菌属（*Stenotrophomonas*）、溶杆菌属（*Lysobacter*）、*Rheinheimera*、*Cobetia*、考克氏菌属（*Kocuria*）、*Oceanobacillus*、*Maribacter*、*Vitellibacter* 和 *Gelidibacter* 共 19 个属的细菌是坛紫菜特有的共附生菌群，未从对照海水中分离到。小红卵菌属（*Rhodovulum*）、假红杆菌属（*Pseudorhodobacter*）、海细菌属（*Marinobacterium*）、*Reinekea*、*Alishewanella*、科尔韦尔氏菌属（*Colwellia*）、*Idiomarina*、戈登氏菌属（*Gordonia*）、微小杆菌属（*Exiguobacterium*）、动球菌属（*Planococcus*）、*Pontibacter*、*Zobellia* 和 *Salinimicrobium* 共 13 个属的细菌仅在对照海水中里分离到。在坛紫菜上和对照海水中都有分布的只有 10 个属的细菌。坛紫菜藻际微环境含有丰富化合物，相比之下海水属于寡营养环境，流动性较大，不同的生长环境很可能导致两者细菌组成的差异。（3）坛紫菜不同养殖时期的细菌存在差异：只在丝状体育苗时期分离到的细菌有 *Sphingopyxis*、假红杆菌属（*Pseudorhodobacter*）、寡养单胞菌属（*Stenotrophomonas*）、*Rheinheimera*、科尔韦尔氏菌属（Colwellia）、*Idiomarina*、*Cobetia*、产丝菌属（*Myceligenerans*）、*Salinibacterium*、戈登氏菌属（*Gordonia*）、考克氏菌属（*Kocuria*）、微小杆菌属（*Exiguobacterium*）、*Zobellia* 和 *Gelidibacter* 共 14 个属，而且归属于放线菌门的细菌基本集中在丝状体时期。只在叶状体海上养殖时期分离到的细菌有 *Phaeobacter*、小红卵菌属（*Rhodovulum*）、*Ahrensia*、*Loktanella*、红杆菌属（*Rhodobacteraceae*）、赤杆菌属（*Erythrobacter*）、*Robiginitomaculum*、芽单胞菌属（*Blastomonas*）、溶杆菌属（*Lysobacter*）、海细菌属（*Marinobacterium*）、*Reinekea*、*Alishewanella*、*Gilvimarinus*、*Oceanobacillus*、*Pontibacter*、*Maribacter*、*Vitellibacter* 和 *Salinimicrobium* 共 18 个属。不同养殖时期，温度、盐度、营养物质、养殖场区的开放性和其他人为干扰在很大程度上影响着细菌群落。此外，同一时期不同的处理方式也使得坛紫菜共附生细菌存在差异。

比较紫菜真菌的分布差异发现（1）分离的真菌大部分集中在丝状体育苗时期，共有 14 个属，叶状体时期只分离到枝顶孢属（*Acremonium*）、枝孢属（*Cladosporium*）、青霉属（*Penicillium*）和曲霉属（*Aspergillus*）共 4 个属的真菌。除曲霉属（*Aspergillus*）是叶状体时期独有的，其他 3 个属是分离真菌数量最多的，为优势菌群。丝状体时期育苗池相对封闭，容易沉积积累有机物，且海水温度、盐度等控制较稳定，为真菌提供了较好地营养基质和生长环境；相比之下叶状体时期海区温度偏低、海水流动性很大以及海水的寡营养性都不利于真菌的生长和聚集。（2）坛紫菜共附生真菌和对照海水真菌存在明显差异：镰孢霉属（*Fusarium*）、*Ophiosphaerella*、茎点霉属（*Phoma*）、裂褶菌属（*Schizophyllum*）和粒毛盘菌属（*Lachnum*）共 5 个属只分离自坛紫菜。粉瘤菌属（*Lycogala*）、节菱孢属（*Arthrinium*）、*Toxicocladosporium*、*Neophaeosphaeria* 和 *Paraphaeosphaeria* 共 5 个属只分离自对照海水。

表 5. 细菌 16S rDNA 序列比对结果

Table 5. Results of bacterial 16S rDNA sequence alignment

Sample groups	Genera
BW1SW	*Pseudorhodobacter*, *Alteromonas*, *Psychrobacter*, *Arthrobacter*, *Salinibacterium*, *Exiguobacterium*, *Halobacillus*, *Planococcus*, *Bacillus*, *Zobellia* and *Cellulophaga*
BW1PhC	*Sulfitobacter*, *Paracoccus*, *Stenotrophomonas*, *Rheinheimera*, *Cobetia*, *Gelidibacter* and *Salinibacterium*
BW2SW	*Idiomarina*, *Psychrobacter*, *Myceligenerans*, *Gordonia* and *Bacillus*
BW2PhC	*Roseivivax*, *Sphingopyxis*, *Myceligenerans*, *Kocuria* and *Bacillus*
BW3SW	*Rhodovulum*, *Rhodobacteraceae*, *Marinobacterium*, *Reinekea* and *Gilvimarinus*
BW3PhS	*Sulfitobacter*, *Phaeobacter*, *Roseivivax*, *Ahrensia*, *Loktanella*, *Rhodobacteraceae*, *Erythrobacter* and *Bacillus*
BW3PhG	*Phaeobacter*, *Ahrensia*, *Loktanella*, *Rhodobacteraceae*, *Paracoccus*, *Erythrobacter*, *Robiginitomaculum*, *Blastomonas*, *Lysobacter*, *Gilvimarinus*, *Bacillus* and *Vitellibacter*
BW4SW	*Rhodovulum*, *Alishewanella*, *Psychrobacter* and *Bacillus*
BW4PhS	*Paracoccus*, *Psychrobacter*, *Arthrobacter*, *Oceanobacillus*, *Halobacillus* and *Cellulophaga*
BW4PhG	*Bacillus*, *Maribacter* and *Cellulophaga*

表 6. 真菌 18S rDNA 序列比对结果

Table 6. Results of fungal 18S rDNA sequence alignment

Sample groups	Genera
BW1SW	*Acremonium*, *Neophaeosphaeria*, *Phaeosphaeria*, *Paraphaeosphaeria* and *Cladosporium*
BW1PhC	*Ophiosphaerella*, *Phoma*, *Phaeosphaeria*, *Cladosporium*, *Penicillium*, *Lachnum* and *Schizophyllum*
BW2SW	*Lycogala*, *Arthrinium*, *Acremonium*, *Toxicocladosporium*, *Cladosporium* and *Penicillium*
BW2PhC	*Fusarium*
BW3SW	*Cladosporium*
BW3PhS	*Penicillium*
BW3PhG	*Acremonium* and *Cladosporium*
BW4SW	*Aspergillus*
BW4PhS	*Cladosporium*
BW4PhG	None

本文对每次采样细菌(图 3 – 图 6)和全部 4 次采样的真菌(图 7)的代表菌株构建系统发育树。

3 讨论

传统的分离方法和 PCR-DGGE 技术是分析微生物群落结构最常用的方法之一[25-26],本研究分离的 467 株细菌归属于变形菌门(Alphaproteobacteria 和 Gammaproteobacteria)、放线菌门(Actinobacteria)、厚壁菌门(Firmicutes)和拟杆菌门(Bacteroidetes),优势菌群为 α-变形菌纲和 γ-变形菌纲,与很多藻类共附生细菌群落研究类似[27-29]。有研究表明细菌群落和藻类之间普遍存在宿主特异性[30]。本研究中有 37 个属的细菌是坛紫菜藻际及海水中特有的,只有芽胞杆菌属(*Bacillus*)、节杆菌属(*Arthrobacter*)、考克氏菌属(*Kocuria*)和动球菌属(*Planococcus*)在条斑紫菜藻际及海水中分离到[18]。有报道称假交替单胞菌会引起紫菜病害[7,11],本研究中未分离到该属细菌,此结果与杨锐等[18]在该海区的研究结果相一致。此外我们还分离到了可能引起条斑紫菜细菌性红烂病的科贝特菌属[3-4]细菌(GenBank 序列号 AY628694.1),相似度高达 99%的菌株。

分离的 55 株真菌大部分归属于子囊菌门(Ascomycota),包括粪壳菌纲(Sordariomycetes)、座囊菌纲(Dothideomycetes)和锤舌菌纲(Leotiomycetes),1 株归属于担子菌门(Basidiomycota)伞菌纲(Agaricomycetes)。枝顶孢属(*Acremonium*)、节菱孢属(*Arthrinium*)、镰孢霉属(*Fusarium*)、茎点霉属(*Phoma*)、枝孢属(*Cladosporium*)、青霉属(*Penicillium*)和曲霉属(*Aspergillus*)是常见的陆生真菌,也普遍存在于海藻中[31-34]。其中,如镰孢霉属(*Fusarium*)、曲霉属(*Aspergillus*)等腐霉具有分解作用,病害发生时藻体常常溃烂解体,这类真菌很可能作为病害的预警因子。在坛紫菜真菌中发现与引起条斑紫菜丝状体白斑病的茎点霉形态极为相似的真菌[12]。据 Zuccaro 和 Mitchell[35]报道,归属于 *Spathulospora*、*Chadefaudia*、*Haloguignardia*、*Retrostium*、*Histopidicarpomyces* 和 *Pontogenia* 的真菌都是藻类特异真菌,而本研究并未分离到相关真菌。此外本研究中坛紫菜真菌的数量和种类明显少于藻际细菌,支持海洋真菌多样性低于细菌多样性的观点[36-38]。

比较微生物群落结构,我们发现坛紫菜藻际微生物和相应海区的海水微生物存在明显差异。同样的情况也出现在绿藻 *Ulva australis* 和 *Ulvacean* sp. 的藻际和海水微生物群落之间[39-40]。这很可能与两者所处环境差异有关。藻类不断向藻际微环境释放各种碳源和营养物质,并且为藻际微生物提供蔽护;而海水中的营养物质浓度低,且海水中的游离微生物还需抵御各种生物与非生物胁迫[39]。另有研究表明海水中的无机营养盐对藻际微生物群落变化无显著影响[41],藻体分泌的化合物可能成为菌群结构的选择性压力[42-45]。此外藻体表面容易形成生物膜,紧密的菌菌互作也可能进一步影响微生物群落结构。Kanagasabhapathy 等[46]从 9 种红藻包括条斑紫菜中分离了 92 株细菌,其中 33% 具有抗菌活性。方文雅[47]、杨锐[18]等也发现紫菜藻际细菌中有 28% 以上菌株具有较强的抑菌活性,而且从健康紫菜上分离的细菌较病烂紫菜或周边海水中分离的

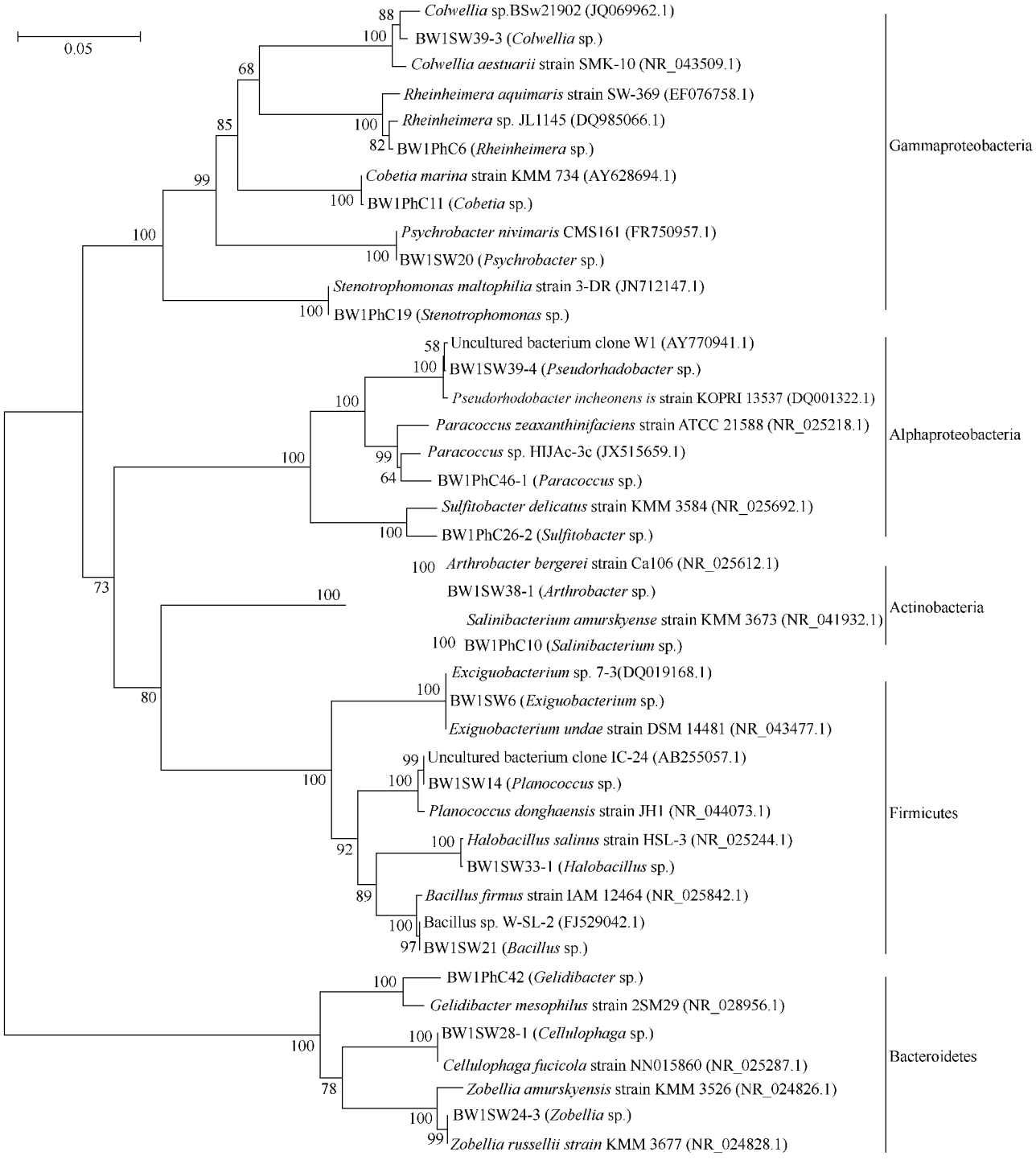

图 3. 2010-04 采样分离细菌的系统发育树

Figure 3. Phylogenetic tree of bacterial isolates sampled in Aprial 2010. The numbers at the nodes indicate the bootstrap values based on neighbor-joining analyses of 1000 sample date sets. The scale bar represents the estimated number of base changes per nucleotide sequence position. Bacterial isolates are indicated with "BW1", and the highest homology species are reflected in parentheses. The others represent stardand strains and the numbers in parentheses are the accession numbers of sequences.

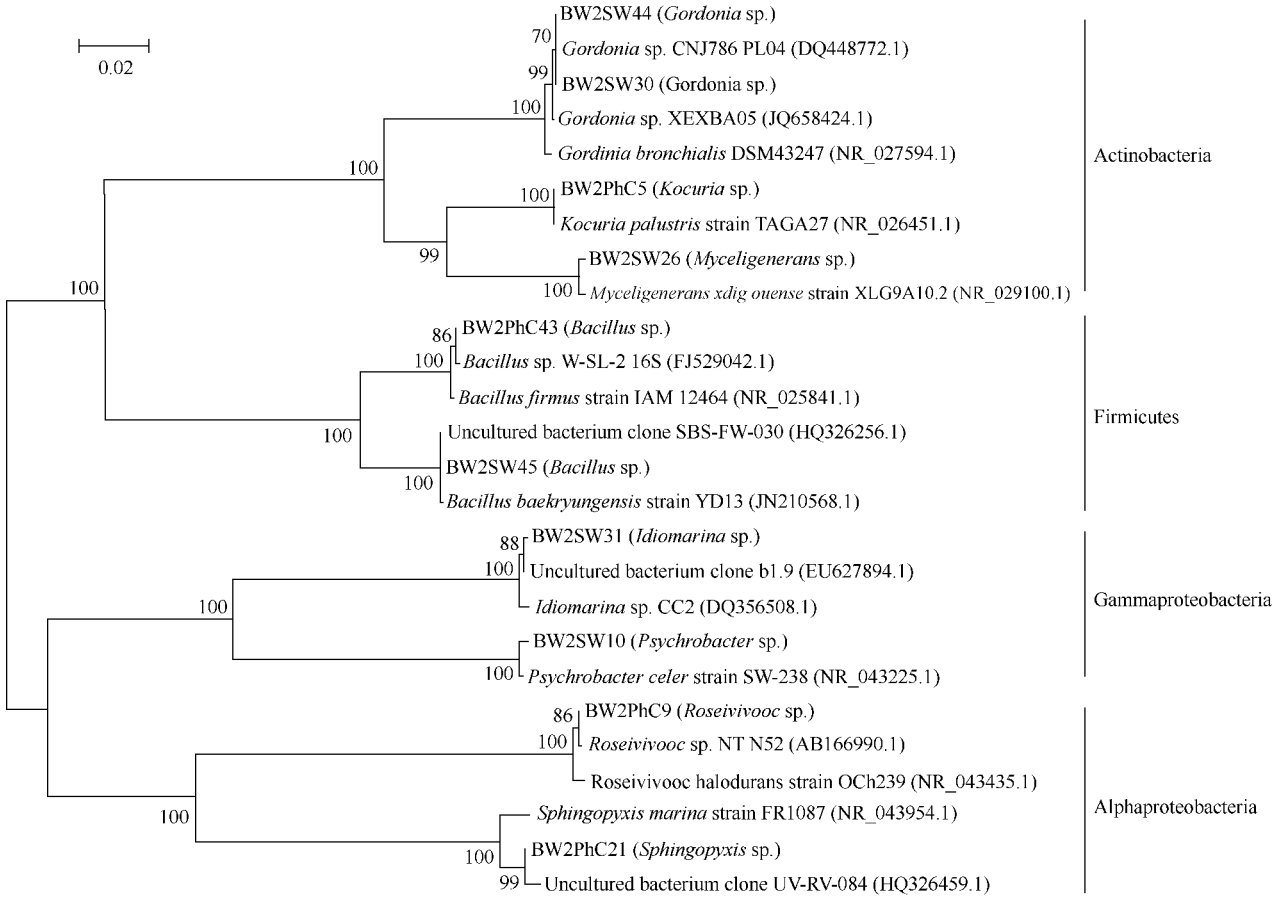

图 4. 2010-09 采样分离细菌的系统发育树

Figure 4. Phylogenetic tree of bacterial isolates sampled in September 2010. The numbers at the nodes indicate the bootstrap values based on neighbor-joining analyses of 1000 sample date sets. The scale bar represents the estimated number of base changes per nucleotide sequence position. Bacterial isolates are indicated with "BW2", and the highest homology species are reflected in parentheses. The others represent stardand strains and the numbers in parentheses are the accession numbers of sequences.

细菌具有更强的抑菌活性和更多的菌株数，这些细菌很可能通过释放抗菌物质抑制其他共生菌以维持自己的生存地位。

坛紫菜丝状体和叶状体阶段藻体形态差异较大，其藻际微生物群落结构也存在较大差异。大部分真菌分离自丝状体。丝状体附着的贝壳微孔同样利于丝状真菌的附着且能提供一定的蔽护作用，叶状体细胞细胞壁较厚，在健康状态下不利于菌丝的侵染。4 个采样时期的海水温度、盐度和 pH 值差异较大(表 2)，这些环境因素也在一定程度上影响微生物群落的变化。丝状体时期苗场的 pH 低于海区 pH，温度基本在 15℃ – 30℃，利于真菌等微生物生长繁殖。叶状体栽培在开放海区进行，海水温度的变化成为较大的选择性压力。叶状体时期海区温度普遍偏低且 pH 接近 8，不利于真菌生长。Dziallas 和 Grossart[15] 研究发现仅不同培养温度下的蓝藻 *Microcystis aeruginosa* 藻际微生物群落明显不同。Tujula 等[40] 研究海洋绿藻 *Ulvacean* sp. 外生细菌群落多样性随地理位置和季节演替，α-变形菌纲和拟杆菌纲作为优势菌群处于较稳定状态，可能发挥着维持群落功能的重要作用。Lachnit 等[30] 研究 *Fucus vesiculosus*、*Gracilaria vermiculophylla* 和 *Ulva intestinalis* 3 种海藻上的外生细菌发现群落结构具有藻类特异性且随季节变化。

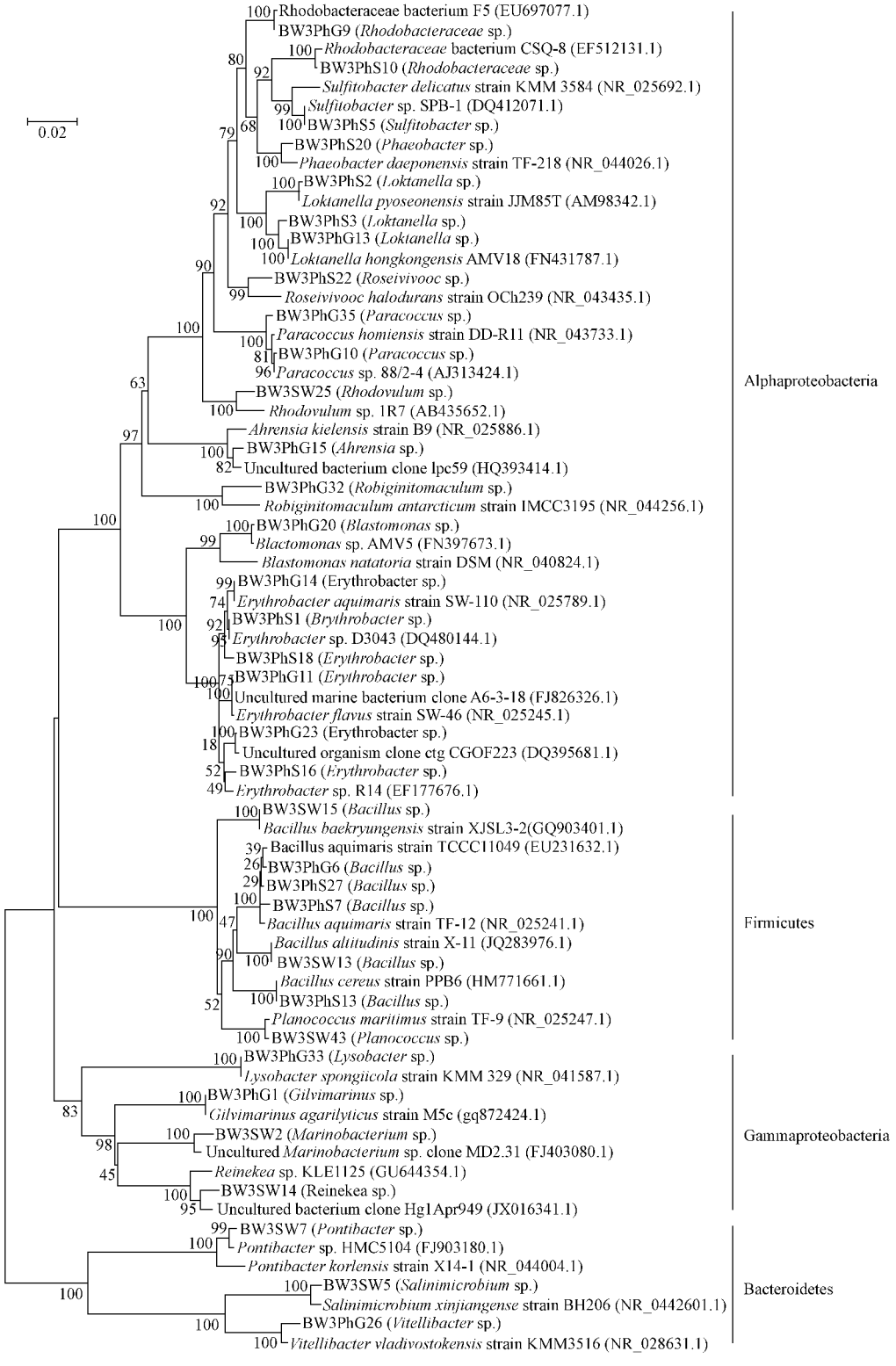

图 5. 2010-11 采样分离细菌的系统发育树

Figure 5. Phylogenetic tree of bacterial isolates sampled in November 2010. The numbers at the nodes indicate the bootstrap values based on neighbor-joining analyses of 1000 sample date sets. The scale bar represents the estimated number of base changes per nucleotide sequence position. Bacterial isolates are indicated with "BW3", and the highest homology species are reflected in parentheses. The others represent stardand strains and the numbers in parentheses are the accession numbers of sequences.

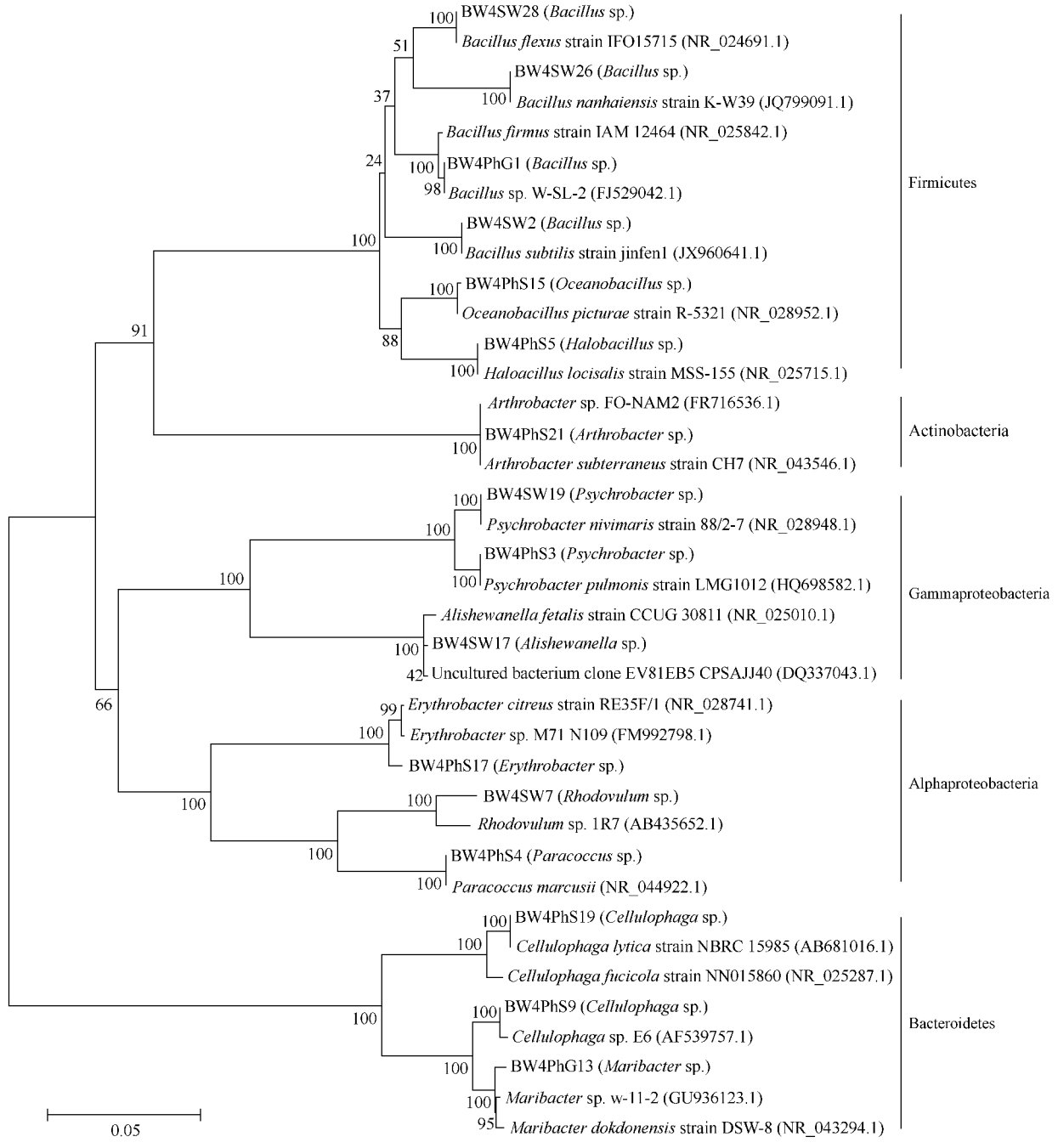

图 6. 2011-03 采样分离细菌的系统发育树

Figure 6. Phylogenetic tree of bacterial isolates sampled in March 2011. The numbers at the nodes indicate the bootstrap values based on neighbor-joining analyses of 1000 sample date sets. The scale bar represents the estimated number of base changes per nucleotide sequence position. Bacterial isolates are indicated with "BW4", and the highest homology species are reflected in parentheses. The others represent stardand strains and the numbers in parentheses are the accession numbers of sequences.

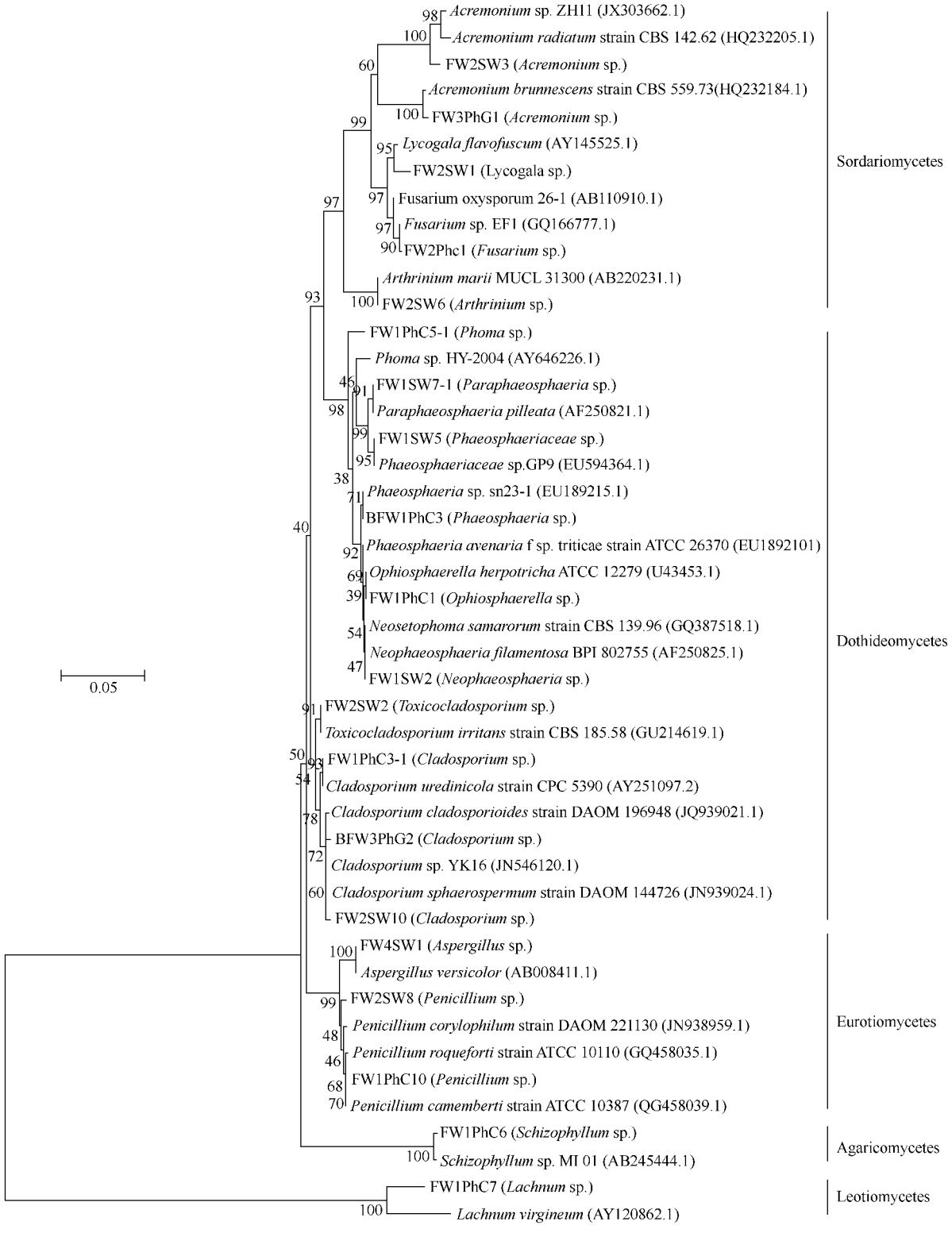

图 7. 4 次采样分离真菌的系统发育树

Figure 7. Phylogenetic tree of fungal isolates in all sampling times. The numbers at the nodes indicate the bootstrap values based on neighbor-joining analyses of 1000 sample date sets. The scale bar represents the estimated number of base changes per nucleotide sequence position. Fungal isolates are indicated with "FW", and the highest homology species are reflected in parentheses. The others represent stardand strains and the numbers in parentheses are the accession numbers of sequences.

综上，坛紫菜藻际和周围海水微生物受宿主和环境影响，虽有发现与致病菌高度相似的微生物，但并未引发病害；可见，良好的藻际微环境能够维持藻类和微生物间的动态平衡，即使有潜在致病微生物的存在也不一定引起藻体病烂。然而，当环境剧变，如高温导致藻类发生生理不适或病烂，个别藻际微生物菌群可能趁机爆发，加剧藻体死亡，继而引发病原性病烂。近年有研究表明结合环境因素和微生物分子生物学分析可以用种群分布模型（species distribution model，SDM）预测微生物多样性[48-49]。坛紫菜养殖虽然有人为控制苗场和天然开放海区两个明显不同的环境，但是这些环境因素均可监测，通过大量环境和微生物数据收集整理，有望绘制坛紫菜养殖周期的微生物生态图。关注正常的藻际微生物群落变化以及可能引起紫菜病害的相关微生物和分解作用的腐霉，在种类和数量上归纳养殖微生物指标，可能最终实现智能化条件控制和病害预防。

参考文献

[1] Sutherland JE, Lindstrom SC, Nelson WA, Brodie J, Lynch MDJ, Hwang MS, Choi HG, Miyata M, Kikuchi N, Oliveira MC, Farr T, Neefus C, Mols-Mortensen A, Milstein D, Müller KM. A new look at an ancient order: generic revision of the Bangiales (Rhodophyta). *Journal of Phycology*, 2011, 47(5): 1131-1151.

[2] Luo Q, Xu Z, Wang C. Effect of high temperature on seedling conchocelis of *Porphyra haitanensis*. *Journal of Ningbo University* (NSEE), 2004, 17(4): 393-396. (in Chinese)
骆其君，徐志标，王常青. 高温时期坛紫菜纯系育苗的对策. 宁波大学学报(理工版)，2004，17(4)：393-396.

[3] Yan X, Huang B, Zhou X, Li L. Study on a bacterial red-rotting disease of *Porphyra haitanensis* (Bangiales, Rhodophyta). *Journal of Fishery Sciences of China*, 2008, 15(2): 313-319. (in Chinese)
严兴洪，黄林彬，周晓，李琳. 坛紫菜叶状体的细菌性红烂病研究. 中国水产科学，2008，15(2)：313-319.

[4] Huang B, Yan X. Study on the redrotting disease of *Porphyra* blades. *Journal of Shanghai Ocean University*, 2010, 19(2): 226-231. (in Chinese)
黄林彬，严兴洪. 紫菜叶状体的红烂病研究. 上海海洋大学学报，2010，19(2)：226-231.

[5] 陈马马，于义德，杨肇惠. 栽培紫菜叶状体绿烂病因的研究//中国藻类学会. 第一届中国藻类学术讨论会论文集. 北京：科学出版社，1983：35-44.

[6] Ding H. Study on development process of green spot disease in *Porphyra yezoensis*. *Journal of Anhui Agricultural Sciences*, 2008, 36(11): 4626-4628. (in Chinese)
丁怀宇. 条斑紫菜绿斑病发病进程研究. 安徽农业科学，2008，36(11)：4626-4628.

[7] Yan Y, Ma J, Xu P, Sun Q, Wang H. *Pseudoalteromonas citrea*, the causative agent of green-spot disease of *Porphyrae yezoensis*. *Journal of Fishery Sciences of China*, 2002, 12, 9(4): 353-358. (in Chinese)
闫咏，马家海，许璞，孙其焕，王汉清. 1 株引起条斑紫菜绿斑病的柠檬假交替单胞菌. 中国水产科学，2002，12，9(4)：353-358.

[8] Takahashi M, Ichitani T, Sasaki M. *Pythium porphyrae* Takahashi et Sasaki, sp. nov. causing red rot of marine red algae *Porphyra* spp.. *Transactions of the Mycological Society of Japan*, 1977, 18(3): 279-285.

[9] Sekimoto S, Yokoo K, Kawamura Y, Honda D. Taxonomy, molecular phylogeny, and ultrastructural morphology of *Olpidiopsis porphyrae* sp. nov. (Oomycetes, straminipiles), a unicellular obligate endoparasite of Bangia and *Porphyra* spp. (Bangiales, Rhodophyta). *Mycological Research*, 2008, 112(3): 361-374.

[10] Klochkova TA, Shim JB, Hwang MS, Kim GH. Host-parasite interactions and host species susceptibility of the marine oomycete parasite, *Olpidiopsis* sp., from Korea that infects red algae. *Journal of Applied Phycology*, 2012, 24(1): 135-144.

[11] Wang H, Li X, Xia Y, Yan B. Isolation, identification and biological pathogen of yellow spot disease in conchocelis of *Porphyra yezoensis*. *Marine Environmental Science*, 2011, 30(30): 361-364, 408. (in Chinese)
王洪斌，李信书，夏亚明，阎斌伦. 条斑紫菜丝状体黄斑病病原体分离鉴定及生物学特性研究. 海洋环境科学，2011，30(30)：361-364，408.

[12] 李金波. 条斑紫菜丝状体白斑病病原菌的分离鉴定. 华中农业大学的学位论文，2006.

[13] Bell W, Mitchell R. Chemotactic and growth responses of marine bacteria to algal extracellular products. *The Biological Bulletin*, 1972, 143(2): 265-277.

[14] Weinberger F. Pathogen-induced defense and innate

immunity in Macroalgae. *Biological Bulletin*, 2007, 213 (3): 290-302.

[15] Dziallas C, Grossart HP. Microbial interactions with the cyanobacterium *Microcystis aeruginosa* and their dependence on temperature. *Marine Biology*, 2012, 159 (11): 2389-2398.

[16] Berg GM, Repeta DJ, LaRoche J. Dissolved organic nitrogen hydrolysis ates in axenic cultures of *Aureococcus anophagefferens* (Pelagophyceae) comparison with heterotrophic bacteria. *Applied and Environmental Microbiology*, 2002, 68 (1): 401-404.

[17] Chen G, Shen H, Zhu M, Xu P. Preliminary investigation on attaching bacteria and disease bacteria in thalli of *Porphyra yezoensis*. *Journal of Aquaculture*, 1999 (1): 17-19. (in Chinese)
陈国耀, 沈怀舜, 朱庙先, 徐璞. 条斑紫菜叶状体附生菌及病原菌初步调查. 水产养殖, 1999 (1): 17-19.

[18] Yang R, Fang W, Shan Y, Chen H, Sun X, Ye Y. Genetic diversity of epiphytic bacteria in *Porphyra yezoensis*. *Acta Oceanologica Sinica*, 2008, 30 (4): 161-168. (in Chinese)
杨锐, 方文雅, 单媛媛, 陈海敏, 孙雪, 叶央芳. 条斑紫菜外生细菌的遗传多样性. 海洋学报, 2008, 30 (4): 161-168.

[19] Xiang T, Zhang F. Microbial diversity in petroleum reserviors analyzed by PCR-DGGE. *Acta Ecologica Sinica*, 2005, 25 (2): 237-242. (in Chinese)
向廷生, 张凡. PCR-DGGE 方法分析原油储层微生物群落结构及种群多样性. 生态学报, 2005, 25 (2): 237-242.

[20] Vainio EJ, Hantula J. Direct analysis of wood-inhabiting fungi using denaturing gradient gel electrophoresis of amplified ribosomal DNA. *Mycological Research*, 2000, 104 (8): 927-936.

[21] Dar SA, Kuenen JG, Muyzer G. Nested PCR-denaturing gradient gel electrophores is approach to determine the diversity of sulfate-reducing bacteria in complex microbial communities. *Applied and Environmental Microbiology*, 2005, 71 (5): 2325 -2330.

[22] Holmström C, Kjelleberg S. Marine Pseudoalteromonas species are associated with higher organisms and produce biological active extracellular agents. *FEMS Microbiology Ecology*, 1999, 30 (4): 285-293

[23] Schwarzott D, Schüler A. A simple and reliable method for SSU rRNA gene DNA extraction, amplification and cloning from single AM fungal spore. *Mycorrhiza*, 2001, 10 (4): 203-207.

[24] Gong L, Ren N, Xing D. Application of denaturing gradient gel electrophoresis and temperature gradient gel electrophoresis in microbial molecular ecology. *Acta Microbiologica Sinica*, 2004, 44 (6): 845-848. (in Chinese)
宫曼丽, 任南琪, 邢德峰. DGGE/TGGE 技术及其在微生物分子生态学中的应用. 微生物学报, 2004, 44 (6): 845-848.

[25] Sun S, Zhang D, Qian L, Pan Z, Chen W. Comparative investigation of the heterotrophic bacterial community in the surface sediment of *Portunus trituberculatus* rearing pond. *Journal of Fisheries of China*, 2010, 34 (5): 820-828. (in Chinese)
孙苏燕, 张德民, 钱丽君, 潘志崇, 陈文桂. 三疣梭子蟹养殖塘表层底泥异养细菌群落比较研究. 水产学报, 2010, 34 (5): 820-828.

[26] Qian L, Zhang D, Xu X. Bacteria diversity in *Portunus trituperculatus* pond sediment estimated by denaturing gradient gel electrophoresis. *Journal of Fisheries of China*, 2007, 31 (2): 204-210. (in Chinese)
钱丽君, 张德民, 徐小红. 应用 DGGE 分析三疣梭子蟹养殖塘底泥细菌的多样性. 水产学报, 2007, 31 (2): 204-210.

[27] Goecke F, Thiel V, Wiese J, Labes A, Imhoff JF. Algae as an important environment for bacteria-phylogenetic relationships among new bacterial species isolated from algae. *Phycologia*, 2013, 52 (1): 14-24.

[28] Longford SR, Tujula NA, Crocetti GR, Holmes AJ, Holmstrom C, Kjelleberg S, Steinberg PD, Taylor MW. Comparisons of diversity of bacterial communities associated with three sessile marine eukaryotes. *Aquatic Microbial Ecology*, 2007. 48 (3): 217-229.

[29] Lage OM, Bondoso J. Planctomycetes diversity associated with macroalgae. *FEMS Microbiology Ecology*, 2011, 78 (2): 366-375.

[30] Lachnit T, Blumel M, Imhoff JF, Wahl M. Specific epibacterial communities on macroalgae: phylogeny matters more than habitat. *Aquatic Biology*, 2009, 5: 181-186

[31] Kohlmeyer J, Kohlmeyer E. Marine Mycology: The high fungi. New York: Academic Press, 1979: 1-690.

[32] Kohlmeyer J, Volkmann-Kohlmeyer B. Marine ascomycetes from algae and animal hosts. *Bot anica Marina*, 2003, 46 (3): 285-306.

[33] Zuccaro A, Schoch CL, Spatafora JW, Kohlmeyer J,

Draeger S, Mitchell JI. Detection and identification of fungi intimately associated with the brown seaweed *Fucus serratus*. *Applied and Environmental Microbiology*, 2008, 74(4): 931-941.

[34] Ding L, Qin S, Li F, Chi X, Laatsch H. Isolation, antimicrobial activity, and metabolites of gungus *Cladosporium* sp. associated with red alga *Porphyra yezoensis*. *Current Microbiology*, 2008, 56(3): 229-235.

[35] Zuccaro A, Mitchell JI. Fungal communities of seaweeds // Deighton J, White JF, Oudemans P. The Fungal Community: Its organisation and role in the ecosystem. 3rd eds. New York: Taylor & Francis, 2005: 533-580.

[36] Kis-Papo T. Marine fungal communities // Dighton J, White JF, Oudemans P. The Fungal Community: Its organisation and role in the ecosystem. 3rd eds. New York: Taylor & Francis, 2005: 61-92.

[37] Burgaud G, Le Calvez T, Arzur D, Vandenkoornhuyse P, Barbier G. Diversity of culturable marine filamentous fungi from deep-sea hydrothermal vents. *Environmental Microbiology*, 2009, 11(6): 1588-1600

[38] Richards TA, Jones MDM, Leonard G, Bass D. Marine fungi: their ecology and molecular diversity. *Annual Review of Marine Science*, 2012, 4: 495-522.

[39] Tujula NA, Crocetti GR, Burke C, Thomas T, Holmström C, Kjelleberg S. Variability and abundance of the epiphytic bacterial community associated with a green marine *Ulvacean* alga. *The ISME Journal*, 2010, 4: 301-311.

[40] Burke C, Thomas T, LewisM, Steinberg P, Kjelleberg S. Composition, uniqueness and variability of the epiphytic bacterial community of the green alga *Ulva australis*. *The ISME Journal*, 2011, 5: 590-600.

[41] Sapp M, Schwaderer AS, Wiltshire KH, Hoppe HG, Gerdts G, Wichels A. Species-specific bacterial communities in the phycosphere of microalgae? *Microbial Ecology*, 2007, 53(5): 683-699.

[42] Lam C, Stang A, Harder T. Planktonic bacteria and fungi are selectively eliminated by exposure to marine macroalgae in close proximity. *FEMS Microbiology Ecology*, 2008, 63(3): 283-291.

[43] Salaün S, Barre SL, Santos-Goncalvez MD, Potin P, Haras D, Bazire A. Influence of exudates of the kelp on biofilm formation of associated and exogenous bacterial Epiphytes. *Microbial Ecology*, 2012, 64(2): 359-369.

[44] Goecke F, Labes A, Wiese J, Imhoff JF. Dual effect of macroalgal extracts on growth of bacteria in Western Baltic Sea. *Revista de Biología Marinay Oceanografía*, 2012, 47(1): 75-86.

[45] Goecke FR, Labes, A, Wiese J, Imhoff JF. Chemical interactions between marine macroalgae and bacteria. *Marine Ecology Progress Series*, 2010, 409: 267-299.

[46] Kanagasahapathy M, Sasaki H, Nagata S. Phylogenetic identification of epibiotic bacteria possessing antimicrobial activities isolated from red algal species of Japan. *World Journal of Microbiology and Biotechnology*, 2008, 24(10): 2315-2321.

[47] Fang W, Yang R, Shan Y, Yan X. Antibacterial activity of *Porphyra* spp. epiphytic bacteria and polyketide synthase I gene screening. *Acta Microbiologica Sinica*, 2009, 49(2): 153-160. (in Chinese)
方文雅, 杨锐, 朱鹏, 单媛媛, 严小军. 紫菜外生细菌抑菌活性及其多聚酮合酶(PKSI)基因筛选. 微生物学报, 2009, 49(2): 153-160.

[48] Fierer N, Ladau J. Predicting microbial distributions in space and time. *Nature Methods*, 2012, 9(6): 549-551.

[49] Larsen PE, Field D, Gilbert JA. Predicting bacterial community assemblages using an artificial neural network approach. *Nature Methods*, 2012, 9(6): 621-625.

Microbial diversity of *Pyropia haitanensis* phycosphere during cultivation

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Abstract: [**Objective**] *Pyropia haitanensis* is of great commercial importance and wildly cultivated in Zhejiang and Fujian provinces. To observe the characteristics and changes of phycosphere microbial communities during cultivation can help us monitor the potential pathogens and microbial factors affecting the health of cultivated seaweeds. [**Methods**] The morphological characteristics and the diversity of phycosphere and surrounding seawater microbes were studied by pure culture method and polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE). Similarity analysis was carried out online with the 16S rDNA (bacteria) and 18S rDNA (fungi) sequences in GenBank. The phycosphere microbial diversity during different growth stages, cultivated areas and periods was studied. [**Results**] Totally 467 bacteria and 55 fungi were isolated during *P. haitanensis* cultivation. The diversity of fungi was smaller than that of bacteria. The bacteria were classified into 41 genera, belonging to Alphaproteobacteria, Gammaproteobacteria, Actinobacteria, Firmicutes and Bacteroidetes. The dominant bacterial communities were Alphaproteobacteria and Gammaproteobacteria. Most of the fungi were classified into Ascomycota, only one strain belonging to the Basidiomycota, Agaricomycetes. Bacteria of 19 specific genera were isolated from *P. haitanensis* while 13 specific genera were isolated from the surrounding seawater. Most actinomycetes and fungi were isolated from the conchocelis cultured indoors, which was different from the microbial communities of the thalli in intertidal zone. Within the isolated microbes, we found that some strains had very high similarity with those pathogens such as *Cobetia marina* (*C. marina*, *P. haitanensis* red-rotting disease), *Phoma porphyrae* (*P. yezoensis* disease) and saprotrophic fungi *Fusarium* sp. and *Aspergillus* sp.. [**Conclusion**] The diversity of *Pyropia* phycoshpere microbes during cultivation was affected by the seaweed morphology, culture time and environmental factors. The strains that shared high similarity with *Pyropia* pathogens were found in this study, which would cause our great attention to these potential pathogens for *Pyropia* diseases.

Keywords: *Pyropia haitanensis*, bacteria, fungi, diversity, cultivation

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