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Research Paper 研究报告

Seasonal and spatial variation of Deuteromycetes population in polluted cost of Kiaochow Bay

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Abstract: [**Objective**] To reveal the relationship between Deuteromycetes community and the environmental in Kiaochow Bay of the Yellow Sea. [**Methods**] Using recorded pollution survey, we used molecular methods to study seasonal and spatial variation of Deuteromycetes community diversity in different polluted waters of Kiaochow Bay of the Yellow Sea, China. [**Results**] Denaturing gradient gel electrophoresis fingerprints varied obviously among different sites of similar level of pollution. Moreover, sequence analysis of recovered dominant bands exhibited the existence of plenty of uncultivable fungi, among which *Penicillium* was the dominant genus. Furthermore, in heavily polluted estuary, there were abundant animal pathogens such as amoeba and *Pythium* as well as Deuteromycetes. These discoveries demonstrate that the Deuteromycetes community structure is closely related to marine environment, and are indicative of different level of marine contamination. [**Conclusion**] The relationship between Deuteromycetes community and different level of pollution and seasons varied were closely related.

Keywords: polluted marine area, deuteromycetes, PCR-DGGE, temporal and spatial diversity

With the rapid economic development and population increasing, marine and coastal areas are under great environmental and ecological pressure due to the discharge of pollutants and irrational exploitation. As a result, the monitoring and control of marine contamination has become an urgent requirements. Meanwhile, microbial community diversity has drawn increasing attention in marine ecological research since the variation of microorganism diversity is closely related to their habitats. However, most of the researches on microbial diversity focused on prokaryotic microorganisms (bacteria) while the diversity of eukaryotic microorganism especially Deuteromycetes in contaminated marine areas was rarely reported. Deuteromycetes are one of the most extensively distributed fungi in marine environment and can even thrive in severe pollution. They are susceptible to the change of marine environment and

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those dominant species can form a new ecological equilibrium, which in turn may have an impact on their environment. In other words, the variation of Deuteromycetes community structure can reflect the level of pollution of their habitats indirectly[1–2]. Although our previous investigation about three randomly selected marine areas of varied pollution levels in the Yellow Sea has roughly demonstrated the relationship between Deuteromycetes group structure and their habitats[2], it should also be noted that coastal marine environments are highly complex because they are under not only the influence of seasonal variation and tidal motion but also some human activities such as the discharge of industrial pollutants. With the rapid development of molecular biology, several research methods were introduced to investigate microbial diversity. In particular, the PCR-DGGE technique can help to detect and analyze multiple samples from complex environments simultaneously, visibly present the microbial community structure and reflect their dynamic spatial and temporal changes, making up for certain deficiencies of traditional isolation-culture methods[3–4]. In order to overcome the shortcomings of single analysis method and to obtain objective results, PCR-DGGE fingerprint technique combined with traditional isolation-culture method were employed in our research to investigate the dynamic variation regularity between Deuteromycetes group structure and environmental contamination in four seasons.

1 Materials and methods

1.1 Determination of sample sites and sampling

Located on the southern coast of the Shandong Peninsula in China and opening to the Yellow Sea, Kiaochow Bay (35°18′N–36°18 N′, 120°04′E–120°23′E) is a semi-closed bay with the total area of 423 km^2 and an average water depth of 7 m. Its river branches such as Haibo River, Licun River and Loushan River are susceptible to pollution by industrial sewage and domestic sewage (Li et al, 2009). According to the results of our previous survey, Licun River (LC) Estuary (36°09′N, 120°21′E) and Haibo River (HB) Estuary (36°06′N, 120°19′E) were severly and moderately polluted respectively while Shilaoren (SL) tidal zone (36°04′N, 120°27′E) was slightly polluted and was selected as the control zone[2]. These three estuaries were set as sampling positions and at each position, four surface layers of coastal sediment (0–20 cm) samples were collected from different sampling sites which were distributed around the center of the estuary and were about 30 m away from each other. Totally 48 samples were collected in spring (April), summer (July), autumn (October) and winter (December). Then, the sediments were put into sterilized sampling bottles, transported to the laboratory immediately and stored at a low temperature for further analysis.

1.2 Environmental contamination indexes analysis

Heavy metals such as Hg, Cu, Zn, Cd and Pb were measured by ICP-MS[5], total nitrogen and total phosphorus were measured by UV-Vis spectrophotometry and ion chromatography[6] and polycyclic aromatic hydrocarbons (PAHs) were measured by GC-MS[7]. Comprehensive pollution index was evaluated according to China Sediment Quality Standard (GB18668-2002)[8] and Marine Investigation Standard (China State Oceanic Administration).

1.3 DNA extraction and purification

Genomic DNA of coastal sediments was

extracted following the protocols described by Zhou et al[9].

1.4 PCR amplification and DGGE fingerprint

Conservative Deuteromycetes 18S rRNA sequences with the length of about 350 bp were amplified by PCR using NS1&GC-Fung primers with GC clamp[10]. PCR amplifications were performed in a 50 μL reaction volume with 5 μL 10× buffer, 1 μL dNTPs (100 mmol/L), 1 μL of each primer, 3 μL $MgCl_2$, 1 μL template (DNA extracted from sediment), 2.5 U of *Taq* DNA polymerase. The PCR conditions were as follows: 5 min of initial denaturation at 94 °C, 32 cycles at 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2min; followed by an extension of 10 min at 72 °C. The PCR products were detected by agarose gel electrophoresis with the staining of ethidium bromide (10 μg/mL) for 15 min. In order to get sufficient PCR products for subsequent DGGE, each PCR for a specific sample was repeated 3 times and the PCR products were combined into a 1.5 mL centrifuge tube for condensation. 6 μL NaCl (5 mol/L) and 300 μL 100% cold ethanol were added into the above centrifuge tube and centrifuged at 10,000×g for 5 min. After atmospheric drying, the pellet was resuspended in 20 μL sterile deionized water and stored at –20 °C.

DGGE was performed using a D-code system (Bio-Rad Laboratories, Hercules, CA, USA) with PCR products (40 μL) being electrophoresed into an 8% polyacrylamide gel with the depth of 1 mm containing a linear denaturing gradient of 15%–50%. Electrophoresis was carried out by using a TAE running buffer (20 mmol/L Tris-HCl, 10 mmol/L acetic acid, 0.5 mmol/L EDTA, pH 8.0) for 20 h at 50 V and 60 °C. The gels were stained with ethidium bromide (10 μg/mL) and photographed with Gel DocTM EQ (Bio-Rad Laboratories, Hercules, CA, USA).

Digitized DGGE images were analyzed with Quantity One image analysis software (version 4.0, Bio-Rad, USA). UPGMA cluster analysis was performed based on the Pearson similarity, DGGE band position and intensity. Simpson index ($D=1-\Sigma Pi2$) was calculated to present Deuteromycetes diversity of each sample site[11].

1.5 Cloning, sequencing and construction of phylogenetic tree

The dominant DGGE bands were cut and recovered from the gel. The obtained DNA was re-amplified as before with NS1&Fung primers (with no GC clamp). PCR products purified by purification kit (OMEGA) were linked to pGEM-TEasy (Promega) and transformed into competent *E. coli* DH5α. Positive clones were screened and sequenced and all sequences were submitted to GenBank. Related 18S rRNA sequences available on the NCBI website were screened through BLASTN program and then phylogenetic trees were constructed according to genetic distances through ClustW[12] and MEGA 3.1 program, using Neighbor joining method (NJ) with repetitive computation (Bootstraps) 1000 times[13].

2 Results

2.1 Comprehensive pollution test

The contents of contaminants at each specific sample site in four seasons are shown in table 1.

With regard to spatial distribution, the contents of contaminants especially Cu, Zn and Cd in different sampling positions varied notably and were in an increasing sequence from SL, HB to LC. To be specific, the content of Cu, Zn and Cd in HB and LC

Table 1. Contamination index four seasons

Seasons	Sites	Pb/(mg/kg)	Cu/(mg/kg)	Zn/(mg/kg)	Cd/(μg/kg)	Hg/(μg/kg)	PAHs/(ng/g)	TP*/(mg/g)	TN*/(mg/g)
Spring	SL1	15.88	4.92	63.36	120.00	13.09	153.70	0.50	0.51
	SL2	15.02	5.79	39.70	39.35	18.71	140.10	0.39	0.76
	SL3	14.52	4.38	28.57	69.50	12.62	91.30	0.43	1.44
	SL4	18.34	5.65	77.84	24.00	12.05	72.90	1.34	0.43
	HB1	39.32	19.76	75.61	820.00	22.83	184.70	2.69	2.67
	HB2	69.92	15.98	327.9	940.30	16.59	144.30	3.91	0.90
	HB3	32.37	53.01	160.10	516.00	15.55	104.70	2.21	1.22
	HB4	58.11	45.01	289.10	440.90	18.62	220.70	1.20	1.09
	LC1	66.08	74.90	1176.00	1211.00	44.70	139.60	5.23	1.47
	LC2	89.07	74.76	533.90	303.30	24.59	575.80	0.88	2.46
	LC3	62.28	56.14	291.00	1220.00	19.05	190.20	0.32	0.33
	LC4	82.83	96.68	505.80	876.10	26.76	708.10	5.79	1.38
Summer	SL1	20.14	8.89	23.04	204.00	14.12	304.58	1.75	1.75
	SL2	18.98	4.85	48.02	190.00	13.58	128.03	0.77	0.87
	SL3	18.29	6.09	21.46	119.60	11.93	128.03	0.22	0.83
	SL4	22.70	7.11	37.74	89.00	16.62	293.57	0.11	0.43
	HB1	41.53	72.63	272.30	854.70	14.41	247.39	1.45	6.60
	HB2	57.91	12.59	228.60	664.00	17.35	123.31	3.70	5.89
	HB3	73.25	12.11	396.10	500.20	22.43	632.69	2.30	7.28
	HB4	57.93	95.11	310.00	366.00	16.92	146.57	4.30	6.91
	LC1	45.10	78.20	395.70	852.40	44.14	75.27	1.02	0.79
	LC2	80.89	49.26	515.60	1082.00	125.60	964.57	4.24	1.88
	LC3	48.54	22.88	307.20	280.10	18.00	102.43	2.06	1.59
	LC4	77.70	62.04	649.50	765.50	21.50	95.89	1.82	1.01
Autumn	SL1	20.36	5.59	83.36	23.00	13.66	136.39	0.63	0.43
	SL2	18.65	5.15	142.80	123.00	11.67	180.85	0.41	0.41
	SL3	17.33	4.21	50.13	240.00	11.90	180.96	0.56	0.64
	SL4	19.09	4.73	24.62	50.35	13.00	67.26	0.25	0.37
	HB1	51.74	71.01	194.90	194.60	13.81	244.87	3.60	2.11
	HB2	36.40	36.32	132.40	529.30	14.74	304.40	1.06	4.53
	HB3	35.75	57.60	162.70	280.00	14.21	81.18	1.08	1.01
	HB4	45.97	56.09	183.70	329.80	17.56	236.49	2.48	1.49
	LC1	38.75	20.33	164.70	963.40	37.83	73.95	0.52	1.28
	LC2	64.94	43.35	554.10	546.50	24.47	799.97	4.02	1.39
	LC3	48.54	22.88	307.20	280.10	18.00	102.43	2.06	0.71
	LC4	77.70	62.04	649.50	765.50	21.50	95.89	1.82	0.58
Winter	SL1	6.98	5.04	15.30	23.00	13.66	56.90	0.12	0.43
	SL2	8.62	6.41	16.31	123.00	11.67	40.27	0.30	2.96
	SL3	7.95	22.10	19.03	240.00	11.90	79.68	0.49	1.30
	SL4	9.71	3.57	15.04	50.35	13.00	65.60	0.07	0.67
	HB1	31.27	32.40	93.19	194.60	13.81	246.70	0.93	0.90
	HB2	22.14	17.70	55.22	529.30	14.74	89.45	0.84	0.84
	HB3	26.00	49.96	175.00	280.00	14.21	130.34	1.06	1.06
	HB4	20.53	31.03	80.57	329.80	17.56	110.47	0.91	0.59
	LC1	34.11	39.76	142.30	963.40	37.83	882.67	0.68	0.68
	LC2	85.39	25.91	137.60	546.50	24.47	434.25	1.72	1.72
	LC3	43.40	66.88	329.80	280.10	18.00	192.52	2.06	0.36
	LC4	53.70	41.85	286.20	765.50	21.50	85.45	1.16	0.65

TP: total phosphorus; TN: total nitrogen.

were 4–6 times and 7–9 times that of SL respectively. The content of Pb differed the slightest among the three sampling positions with that of HB and LC being 1.3 and 2.5 times of SL respectively.

In terms of seasonal fluctuation, the content of contaminants in SL varied inconspicuously in different seasons while that of HB and LC fluctuated drastically in four seasons. To be specific, total nitrogen in HB fluctuated most wildly with the minimum 0.85 mg/g in winter and the maximum 6.67 mg/g in summer (7.9 times of winter). As for LC, the Zn content fluctuated most distinctly with the maximum of 626.7 mg/kg in spring and the minimum of 418.9 mg/kg in autumn. Despite the variation of contamination indexes according to seasons, there was a general tendency, i.e. there were relatively higher contamination indexes in spring and summer but lower indexes in autumn and winter.

2.2 DGGE fingerprint results

Fungal 18S rRNA was amplified from sediment DNA using the specific primer of marine Deuteromycetes and PCR products were then electrophoresed with linear denaturing gradient gel. The result of each sample exhibited certain amount of bands with varied positions and brightness, which indicted high Deuteromycetes diversity in this marine area (Figure 1-A, B, C, D).

UPMGA cluster analysis of each sampling sites was based on similarity factors calculated according to electrophoresis lanes/bands and the results are shown in Figure 2-A, B, C and D.

It is obvious from Figure 2-A that the 12 spring samples can be classified into three phylogenetic groups. To be specific, the first group comprised SL1, SL2, SL3 and SL4, the second LC1, LC2 and LC3, the third HB1, HB2, HB3 and HB4. However, the fingerprint of LC4 differed distinctly from the rest 11 samples with the similarity being only 14%. The DGGE fingerprints of spring samples between various sites with similar contamination levels in the same sampling position were similar while those between positions of different pollution levels varied apparently. The cluster results of samples in summer, autumn and winter were more complex than those of spring (Figure 2-B, C, D). The DGGE fingerprints between different sites (with a distance of only about 30 m from each other) in the same position such as HB1 and HB2 in summer, LC2 and LC3 in autumn, HB3 and HB4 in winter showed low similarity. Comprehensive pollution results showed evident variation in contaminant content between these specific sites, indicating that the Deuteromycetes community was sensitive to environmental contamination and the change in the levels and types of environmental contamination.

2.3 Diversity index of DGGE bands

Deuteromycetes diversity indexes of samples in different seasons calculated on the basis of the quantity of bands and brightness peak of each DGGE lane are shown in Figure 3. The Deuteromycetes diversity indexes of the three positions varied obviously upon seasonal change.

To be specific, the general trend of Deuteromycetes diversity in SL was summer > autumn > winter > spring with the maximum index of 0.845 in summer and the minimum of 0.695 in spring , while the trend in HB was spring > summer > autumn > winter with the maximum index of 0.865 in spring and the minimum of 0.730 in winter. As for LC, the trend was winter > spring> summer > autumn, which is quite

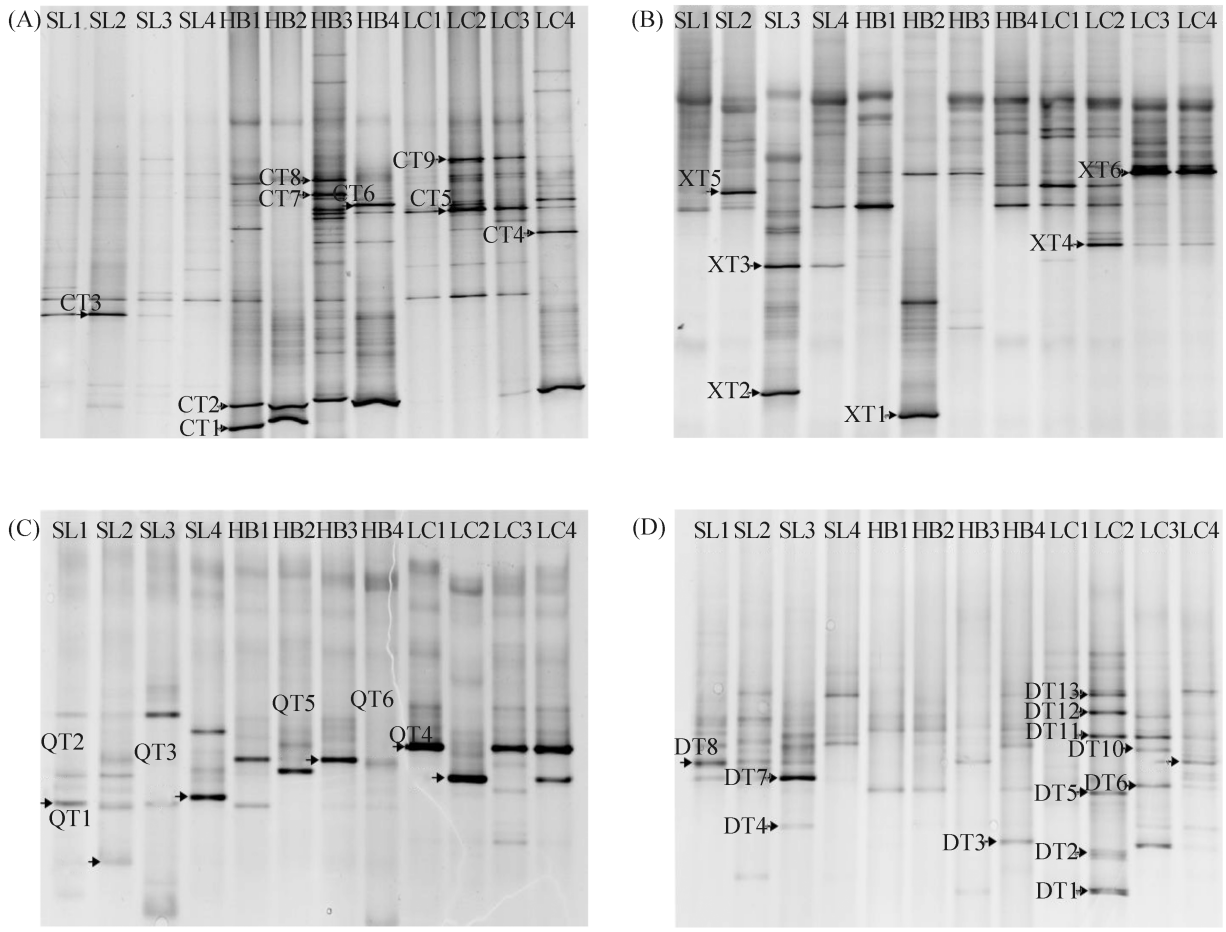

Figure 1. DGGE fingerprint of samples in different seasons. A–D:12 samples in four seasons: spring, summer, autumn and winter.

similar to that of HB except that in LC the diversity index increased to the maximum of 0.878 in winter.

2.4 Excised bands analysis

A total of 34 dominant bands were excised from DGGE gel of four seasons (9 in spring, 6 in summer, 6 in autumn and 13 in winter). Sequential results of the above obtained bands were submitted to GenBank and blasted with other related species to confirm their relationship (Table 2) and generate a phylogenetic tree (Figure4).

Among the 34 excised dominant bands, 12 bands were uncultivable fungi, 8 bands were uncultivable eukaryotes, 5 bands were Deuteromycetes *Alternaria* sp., *Penicillium argillaceum*, *P. phialosporum*, *P. chrysogenum* respectively, 2 bands were zygomycetes phylum *Rhizopus stolonifer* and Mastigomycotina *Pythium insidiosum*, in addition to 7 bands being respectively *Amoebida*, *Stylaria*, *Hartmannella bertawensis*, *Aurearena* and *Mytilus*. According to the sequencing results, most of the dominant bands in DGGE were uncultivable fungi. The dominant bands in slightly polluted SL were mainly uncultivable fungi and *Penicillium chrysogenum* was the dominant species. There were abundant Deuteromycetes species in HB with *Alternaria* sp. and *Penicillium phialosporum as* dominant species. Except for one

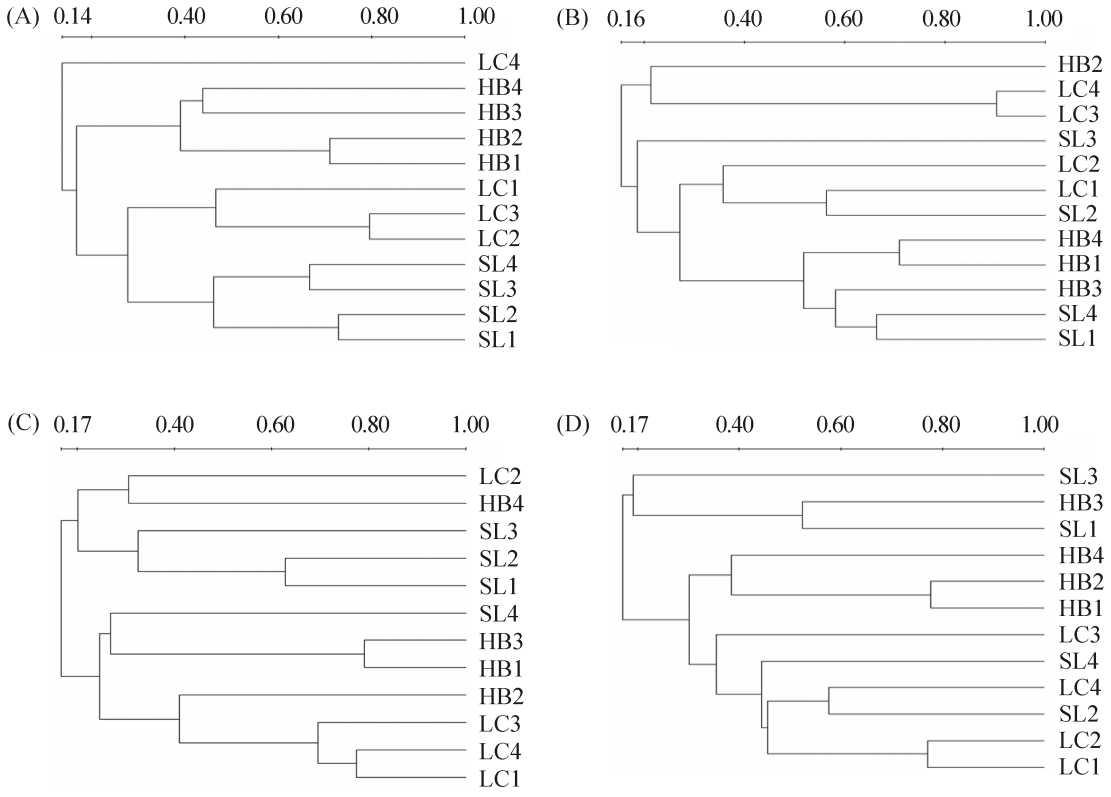

Figure 2. UPMGA cluster analysis of different samples. A–D: 12 samples in four seasons: spring, summer, autumn and winter.

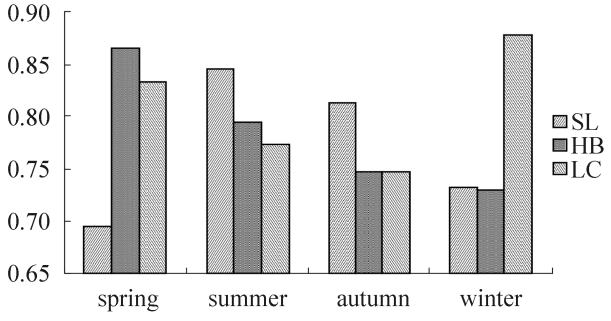

Figure 3. the DGGE bands diversity variation in different seasons.

Deuteromycetes *Penicillium argillaceum*, most of the dominant species in LC were not fungi but primary eukaryotes including unicellular algae *Aurearena*, *Hartmannella bertawensis*, *Rhizopus stolonifer*, and animal pathogens such as uncultivable *Euamoebida*, *Pythium insidiosum,* which further verified the high contamination level of this site.

3 Discussion

Marine ecological environment is faced with serious challenges as a result of industrial development, the discharge of domestic garbage and some environmental incidents such as oil spill. As a semi-closed gulf, Kiaochow Bay is grievously contaminated because of its weak cyclic purifying ability as well as the mass inflow of industrial and domestic sewage which would accumulate and turn into to marine sediments carried by several river branches such as Haibo River, Licun River, Loushan River and Dagu River[14–15]. In general, heavy pollution is comparatively easy to be observed by the disappearance of certain common types of animals and plants and the obvious change in the color of seawater and sediments.

Table 2. Sequence results of excised DGGE bands

Excised bands	Bands origin	Related sequence in GenBank	Accession number	Similarity/%
CT1	HB	*Stylaria* sp.	DQ4599711	98
CT2	HB	uncultivable eukaryote	FJ1537712	95
CT3	SL	uncultivable fungus	GQ8444611	95
CT4	LC	uncultivable fungus	DQ4121311	88
CT5	LC	*Aurearena* sp.	AB3651961	98
CT6	HB	*Alternaria* sp.	U051991	99
CT7	HB	*Alternaria* sp.	AF2187911	99
CT8	HB	uncultivable fungus	AF3727131	88
CT9	LC	*Hartmannella bertawensis*	DQ1902411	95
XT1	HB	uncultivable eukaryote	FJ1537952	92
XT2	SL	uncultivable marine eukaryote	EF5268521	99
XT3	SL	uncultivable fungus	GQ8444611	95
XT4	LC	*Penicillium argillaceum*	FJ2220791	83
XT5	SL	uncultivable fungus	GQ8444671	99
XT6	LC	uncultivable fungus	FN3948441	89
QT1	SL	uncultivable eukaryote	AY1800061	94
QT2	SL	uncultivable fungus	GU0725901	100
QT3	SL	uncultivable fungus	FJ7858761	90
QT4	LC	uncultivable fungus	EU1439851	88
QT5	HB	*Penicillium phialosporum*	AF2452651	82
QT6	LC	*Hartmannella bertawensis*	DQ1902411	95
DT1	LC	uncultivable eukaryote	EU8607471	98
DT2	LC	uncultivable eukaryote	EU8607471	98
DT3	HB	uncultivable fungus	GQ8444611	97
DT4	SL	*Mytilus* sp.	L334521	99
DT5	LC	uncultivable Euamoebida	EU6469451	94
DT6	LC	uncultivable Euamoebida	DQ4121231	94
DT7	SL	*Penicillium chrysogenum*	AM9204311	100
DT8	SL	uncultivable fungus	GQ8444671	99
DT9	LC	*Rhizopus stolonifer*	AB2501761	100
DT10	LC	*Pythium insidiosum*	AF4424971	99
DT11	LC	uncultivable eukaryote	AB2382011	95
DT12	LC	uncultivable eukaryote	EU1440011	94
DT13	LC	uncultivable fungus	FJ2369471	99

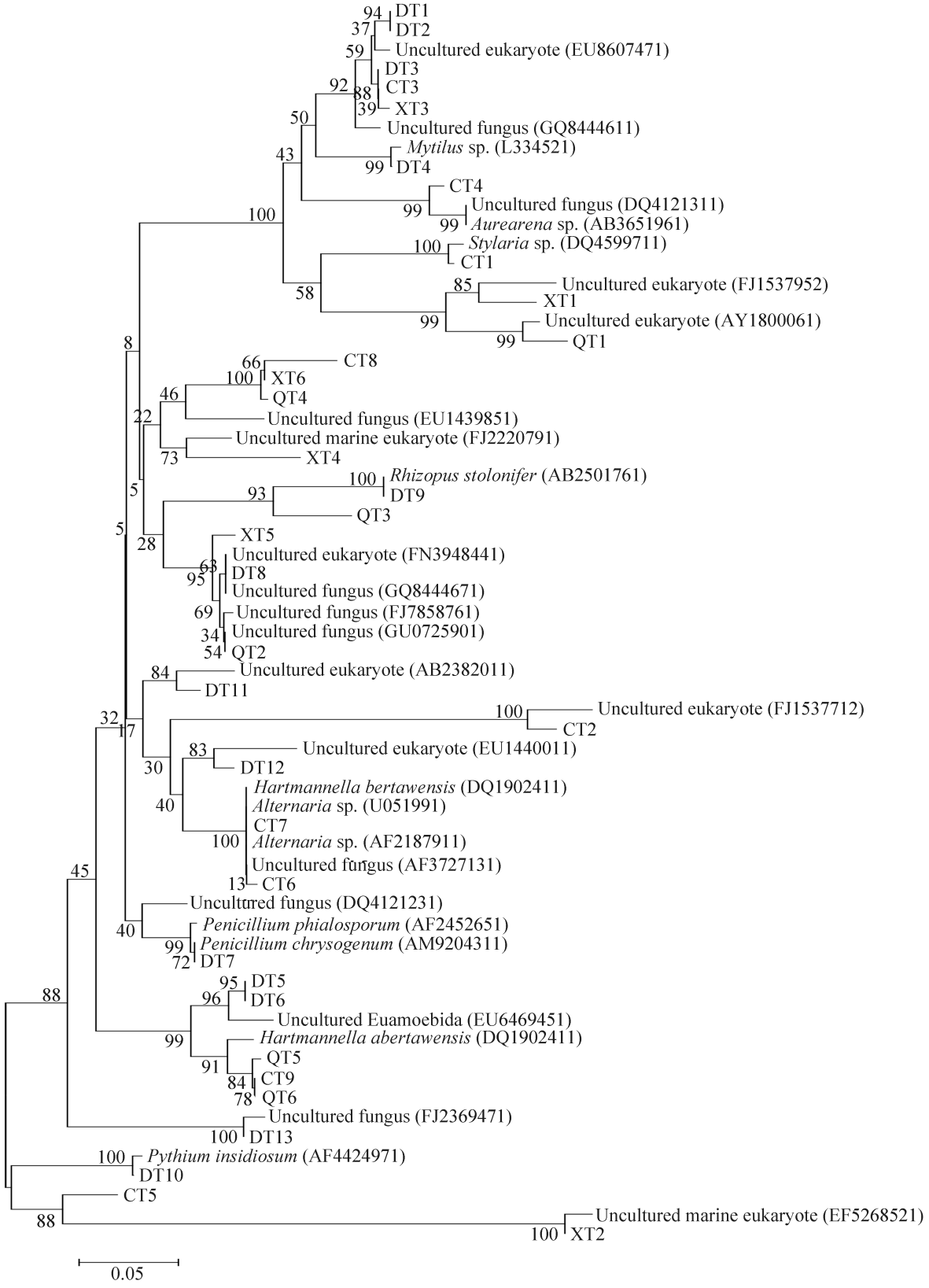

Figure 4. Phylogenetic tree constructed from recovered DGGE bands.

Nevertheless, once this occurs, great environmental damage and immense restoration expenditure are inevitable. In this sense, alert is of significance in the "slight-to-moderate" pollution period since prevention and restoration procedures are can be monitored and pollution may be brought under control.

The variation of biocenose can reflect the change of environment because of the interdependence between biocenose and environment. Lower aquatic animals and plants were utilized worldwide as indicator organisms to monitor marine environment through the accumulation and variation of pollutants in their bodies. Nevertheless, a relatively long period of time is required because the influence of pollutants on organisms starts at the cellular level, and only when pollution reaches a certain stage can individual and ecological effects be observed. Therefore, serious pollution may have already occurred before such organisms begin to show certain abnormity. In this sense, microorganisms are a preferable alternative for detecting marine pollution in early the stage because they are sensitive to environmental changes which could lead to corresponding changes in their community structure and individual pigment and morphologic characteristics. Accordingly, changes of such parameters are indicative of marine pollution in the early stage and our results have confirmed such feasibility.

In order to find out the interaction between Deuteromycetes and marine pollution, the choice of sampling sites is particularly important since marine environment is more complicated than terrestrial environment because of ocean currents. In this research, Haibo River estuary and Licun River estuary of Kiaochow Bay with different pollution levels were selected as sampling sites with Shilaoren as the control area. The comprehensive environmental pollution index through chemical and instrumental analysis was correspondent with previous data, laying the foundation for further Deuteromycetes test.

Currently, most reports on the relationship between marine microorganism diversity and environment have focused on prokaryotic bacteria but research on eukaryotes especially Deuteromycetes have rarely been conducted. Deuteromycetes as well as Ascomycetes are the dominant fungi in marine environment with the latter being usually restrictedly distributed on solid substrate such as float woods, intertidal halophytes and marine algae[16–17]. On the contrary and the former being mostly found in coastal, abyssal and polar marine areas and are especially abundant both in number and species in coastal areas. However, there were neither reports on whether Deuteromycetes existed in comparatively heavily polluted marine area nor research on the relationship between Deuteromycetes community structure and its biotope. In our research, traditional isolation-culture methods (Another article published) combined with modern PCR-DGGE fingerprint technique were adopted to investigate the relationship between seasonal Deuteromycetes community diversity and its biotope. The results demonstrated that Deuteromycetes existed in heavily polluted marine areas where no visible life signs could be found such as Licun River estuary and that the quantity and species of Deuteromycetes in moderately polluted Haibo River estuary unexpectedly exceeded those in clean sites such as Shilaoren marine area. Besides, we found that Deuteromycetes community varied seasonally according to different pollution levels, indicating that Deuteromycetes community

structure was closely related with marine environment and played a certain role in degradation of contaminants or deterioration of environment.

As a powerful means to explore microbial ecology, PCR-DGGE was used in many labs and manifested broad prospect for its unique advantages[18]. However, there are limitations in this method. For example, on the one hand, the microbial diversity is underestimated because it is difficult to extract and amplify all microorganism' genome DNA. Sometimes, when structurally differentiated fragments with coincidently same migration rate might stay in the same position in DGGE gel, the microbial diversity might also be underestimated. On the other hand, the microbial diversity might be overestimated when a single strain presence multiple bands in DGGE gel because 18S rRNA has multiple copies in the chromosome. In order to objectively restore primordial status of Deuteromycetes community structure, a considerable number of cultivable Deuteromycetes were isolated by adjusting selective mediums and through sample pretreatment according to the results of molecular technique and adopting different procedures to restrict terrestrial microorganisms. In addition, many bands of DGGE were uncultivable Deuteromycetes due to the limits of available data in GenBank. Though controversy existed with regard to two analysis methods on Deuteromycetes seasonal variation, it is clear that *Penicillium* was the absolutely dominant genus with both methods. It is also noticeable that *Penicillium expansum* was isolated and cultured in each sample but was not observed in DGGE bands. Hopefully, the follow-up construction of 18S rRNA gene library of this region can explain such phenomenon.

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胶州湾污染海域半知菌群体结构多样性时空动态变化及与生境的关系

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摘要：【目的】揭示胶州湾典型污染海域半知菌群体结构多样性动态变化与生境的关系。【方法】根据以往对近岸污染调查记录，分别确定胶州湾海泊河、李村河入海口为中度、重度污染采样站位，石老人潮间为轻度污染对照站位，利用PCR-DGGE指纹图谱方法，研究该生境半知菌群体结构多样性季节动态变化与污染的关系。【结果】结果证明，污染指标除总氮含量外，其他各项指标含量在三站位的变化趋势为石老人潮间带最低，海泊河居中，李村河含量最高；与石老人站位相比，海泊河、李村河站位污染指标在四季变化较显著。首次证明在重金属和氮磷严重超标的海域，仍存在数量和种类较多的半知菌。PCR-DGGE指纹图谱结果显示，同一站位相似污染程度采样点之间DGGE指纹图谱比较一致，不同站位、不同季节之间指纹图谱存在明显的差异。优势条带割胶测序分析显示，3个站位存在大量的不可培养真菌，青霉属为半知菌优势种，重度污染的李村河入海口除半知菌外，存在较大量的阿米巴虫、腐霉等动物病原菌。【结论】污染海域的半知菌群体结构与生境污染程度及季节具有较密切的联系。

关键词：污染海域，半知菌，PCR-DGGE，时空多样性

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