Supplementary Materials

Algae 2024, 39(3): 177-186
https://doi.org/10.4490/algae.2024.39.8.26
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Supplementary Method S1. Phylogenetic analysis and primer design.

Phylogenetic relationships of *Pythium* strains used in the present study were determined based on *cox*1 and *cox*2 genes as referred previously (Martin 2000, Lee et al. 2015). For the phylogenetic analyses, all sequences were aligned using Clustal W (Thompson et al. 1997). A rooted phylogenetic tree was constructed using the neighbor-joining algorithm in MEGA-X (Kumar et al. 2018). The robustness of the phylogram in the maximum likelihood analysis was evaluated by 1,000 bootstrap replications.

To design the pathogen-specific primers in the patent, the genomes of *P. porphyrae* NBRC No. 30800 and *P. chondricola* NBRC No. 33253 were sequenced (unpublished data) and aligned, which resulted in two pathogen-specific gene fragments with different lengths, i.e., approximately 900 bp and 300 bp, respectively. The two pathogen-specific fragments have been cloned, sequenced, and submitted to GenBank under accession numbers ON158128 and ON158129, respectively. The specificity of the two primer pairs has also been tested using 17 microbial species in the patent, including 12 strains of *Pythium* sp., one strain of *Alternaria* sp., one strain of *Olpidiopsis* sp., and two bacterial strains (Supplementary Table S1). Consequently, the two pathogens can be detected and classified within a single PCR amplification based on product length without sequencing, and hence an attempt to quantify the pathogens using the pathogen-specific primers.

Supplementary Table S1. Microbes used for the specificity test of the pathogen-specific primers

Genera / Species	Strain	Source
Pythium porphyrae	NBRC No. 30800	NBRC
	NBRC No. 33126	NBRC
P. chondricola	HT201801	Our lab
	JS151205	Our lab
	RZ201902	Our lab
	LS201903	Our lab
	NBRC No. 100633	NITE
	NBRC No. 33253	NITE
Other *Pythium* species	*P. ultimum*	Our lab
	P. recalcitrans	CGMCC
	P. inflatum	CGMCC
	P. oopapillum	CGMCC
Others	*Alternaria* sp.	Our lab (Mo et al. 2016)
	Oplidiopsis sp.	Our lab (He et al. 2021)
	Pseudoalteromonas carrageenovora	Our lab
	Bacillus hwajinpoensis	

NBRC, NITE Biological Resource Center; NITE, National Institute of Technology and Evaluation; CGMCC, China General Microbiological Culture Collection Center.

Supplementary Table S2. Coordinates of the sampling sites distributed in Haizhou Bay

Site	Location	2021		2022	
		Dec 16	Dec 23	Jan 6	Feb 24
S1	35°2'57" N, 119°21'30" E	w	ns	w	w
S2	34°59'47" N, 119°17'57" E	w	ns	w	w
S3	35°0'25" N, 119°22'54" E	w	ns	w	w
S4	34°53'46" N, 119°18'2" E	w	ns	w	w
S5	34°53'50" N, 119°26'12" E	w	ns	w	w
S6	34°53'16" N, 119°33'23" E	w	ns	w	w
S7	34°48'38" N, 119°18'50" E	w	ns	w	w
S8	34°48'17" N, 119°24'29" E	w	ns	w	w
S9	34°49'0" N, 119°32'44" E	w	D: w + s UD: w + s	w	w
S10	34°44'30" N, 119°32'44" E	w	ns	w	w

w, seawater sample; s, sediment sample; ns, not sampling.
"D" and "UD" indicate infected and uninfected zone by red rot disease.

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http://e-algae.org pISSN: 1226-2617 eISSN: 2093-0860

Supplementary Table S3. Measurements of environmental parameters in Haizhou Bay

Parameters	Date	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
pH	Dec 16, 2021[b]	8.16 ± 0.02	8.18 ± 0.02	8.16 ± 0.08	8.23 ± 0.04	8.22 ± 0.04	8.17 ± 0.09	8.20 ± 0.03	8.25 ± 0.01	8.23 ± 0.02	8.16 ± 0.04
	Jan 6, 2022[b]	8.32 ± 0.03	8.33 ± 0.02	8.31 ± 0.01	8.32 ± 0.04	8.33 ± 0.03	8.32 ± 0.03	8.20 ± 0.02	8.23 ± 0.03	8.36 ± 0.02	8.36 ± 0.03
	Feb 24, 2022[a]	8.35 ± 0.02	8.36 ± 0.01	8.40 ± 0.01	8.36 ± 0.02	8.35 ± 0.02	8.37 ± 0.02	8.29 ± 0.03	8.32 ± 0.04	8.36 ± 0.04	8.29 ± 0.01
Temperature (°C)	Dec 16, 2021[a]	10.9 ± 0.07	10.1 ± 0.08	11.3 ± 0.19	10.0 ± 0.29	11.8 ± 0.74	13.4 ± 0.71	9.4 ± 0.05	10.0 ± 0.55	10.9 ± 0.61	11.4 ± 0.06
	Jan 6, 2022[b]	5.7 ± 0.23	5.3 ± 0.23	5.8 ± 0.06	6.1 ± 0.06	5.9 ± 0.12	6.2 ± 0.10	4.0 ± 0.06	5.1 ± 0.05	6.0 ± 0.06	6.7 ± 0.04
	Feb 24, 2022[c]	4.5 ± 0.08	4.3 ± 0.06	4.6 ± 0.20	4.2 ± 0.11	5.1 ± 0.15	5.2 ± 0.26	4.0 ± 0.05	4.3 ± 0.21	5.1 ± 0.07	4.7 ± 0.23
Salinity (‰)	Dec 16, 2021[c]	26.95 ± 0.03	26.80 ± 0.33	27.17 ± 0.05	26.17 ± 0.60	27.00 ± 0.53	27.67 ± 0.33	24.94 ± 0.15	26.67 ± 0.57	26.57 ± 0.33	27.66 ± 0.02
	Jan 6, 2022[b]	28.60 ± 0.58	28.35 ± 0.33	28.63 ± 0.02	28.71 ± 0.10	28.4 ± 0.00	28.70 ± 0.20	26.09 ± 0.33	27.58 ± 0.37	28.37 ± 0.18	29.05 ± 0.20
	Feb 24, 2022[a]	29.62 ± 0.31	29.32 ± 0.27	29.60 ± 0.30	28.57 ± 0.58	29.91 ± 0.33	29.88 ± 0.34	26.4 ± 0.40	28.46 ± 0.11	29.75 ± 0.33	29.87 ± 0.23
Dissolved oxygen (%)	Dec 16, 2021[b]	93.03 ± 0.40	94.33 ± 0.04	96.09 ± 0.36	99.48 ± 0.13	106.82 ± 0.52	96.49 ± 0.21	94.63 ± 0.33	110.94 ± 0.03	102.09 ± 0.26	102.30 ± 0.27
	Jan 6, 2022[b]	96.80 ± 0.01	100.10 ± 0.20	98.10 ± 0.26	100.60 ± 0.12	102.50 ± 0.84	98.10 ± 0.02	97.00 ± 0.87	100.80 ± 0.04	102.90 ± 0.05	104.80 ± 0.03
	Feb 24, 2022[a]	108.80 ± 0.39	107.10 ± 0.39	107.00 ± 0.05	103.70 ± 0.40	105.70 ± 0.05	110.50 ± 0.39	103.40 ± 0.08	104.40 ± 0.13	108.40 ± 0.19	99.70 ± 0.09
NH_4^+-N (μg L^{-1})	Dec 16, 2021	5.21 ± 0.14	2.47 ± 0.55	7.01 ± 0.64	2.93 ± 0.39	0.54 ± 0.41	7.04 ± 0.56	19.40 ± 0.15	5.33 ± 0.16	1.12 ± 0.13	0.39 ± 0.08
	Jan 6, 2022	13.48 ± 0.08	4.48 ± 0.19	3.45 ± 0.21	6.93 ± 0.18	151.24 ± 1.04	8.90 ± 0.99	89.45 ± 0.11	14.20 ± 0.70	4.33 ± 0.36	4.19 ± 0.62
	Feb 24, 2022	6.00 ± 0.74	8.16 ± 0.32	4.61 ± 0.05	5.60 ± 0.49	3.67 ± 0.12	3.90 ± 0.33	20.33 ± 0.17	5.54 ± 0.06	8.14 ± 0.26	6.09 ± 0.08
NO_2^--N (μg L^{-1})	Dec 16, 2021[b]	13.06 ± 0.09	13.65 ± 0.05	12.97 ± 0.06	13.98 ± 0.38	13.03 ± 0.42	12.25 ± 0.33	18.84 ± 0.10	15.06 ± 0.11	13.23 ± 0.06	11.20 ± 0.15
	Jan 6, 2022[b]	11.63 ± 0.05	13.42 ± 0.17	12.33 ± 0.20	12.08 ± 0.15	10.67 ± 0.77	9.35 ± 0.10	15.80 ± 0.84	11.05 ± 0.43	11.64 ± 0.21	11.91 ± 0.34
	Feb 24, 2022[a]	21.73 ± 0.20	23.96 ± 0.43	21.55 ± 0.87	25.15 ± 0.33	23.05 ± 0.19	21.28 ± 0.64	32.11 ± 1.11	29.22 ± 1.05	10.02 ± 2.62	10.22 ± 1.28
NO_3^--N (μg L^{-1})	Dec 16, 2021	116.94 ± 5.12	106.35 ± 3.20	106.30 ± 4.23	145.69 ± 5.50	121.60 ± 5.11	97.58 ± 1.51	193.83 ± 4.49	127.70 ± 2.49	124.35 ± 5.28	111.03 ± 1.12
	Jan 6, 2022	61.04 ± 1.12	61.93 ± 1.59	84.62 ± 6.20	63.89 ± 5.12	71.62 ± 3.08	90.10 ± 4.22	178.56 ± 1.60	371.44 ± 3.83	70.66 ± 2.45	66.81 ± 1.71
	Feb 24, 2022	47.30 ± 3.86	50.42 ± 5.21	48.15 ± 5.12	62.46 ± 1.69	47.98 ± 0.46	53.57 ± 5.00	110.92 ± 8.72	61.82 ± 1.91	79.61 ± 1.13	77.18 ± 2.93
PO_4^{3-}-P (μg L^{-1})	Dec 16, 2021	5.98 ± 0.07	3.18 ± 0.06	10.62 ± 0.05	1.65 ± 0.05	9.76 ± 0.13	12.27 ± 0.02	5.78 ± 0.03	1.96 ± 0.05	10.75 ± 0.03	11.85 ± 0.06
	Jan 6, 2022	7.13 ± 0.06	7.55 ± 0.17	8.51 ± 0.15	4.19 ± 0.03	11.57 ± 0.08	7.98 ± 0.03	13.09 ± 0.06	5.72 ± 0.03	10.69 ± 0.11	7.69 ± 0.02
	Feb 24, 2022	3.94 ± 0.09	3.76 ± 0.03	4.36 ± 0.06	4.81 ± 0.05	5.67 ± 0.09	2.92 ± 0.03	6.91 ± 0.44	2.62 ± 0.11	12.65 ± 0.08	9.62 ± 0.03
SiO_3^{2-}-Si (μg L^{-1})	Dec 16, 2021[a]	175.70 ± 1.51	4.33 ± 0.47	257.72 ± 12.57	124.15 ± 0.60	212.47 ± 0.47	260.45 ± 0.42	216.84 ± 2.19	148.53 ± 1.37	216.66 ± 2.50	232.81 ± 2.73
	Jan 6, 2022[b]	115.66 ± 2.77	107.46 ± 1.56	151.58 ± 1.26	106.42 ± 0.97	130.42 ± 0.58	139.56 ± 1.46	207.19 ± 2.71	0.70 ± 1.50	125.60 ± 1.64	143.33 ± 4.11
	Feb 24, 2022[b]	70.58 ± 3.48	69.98 ± 3.76	66.39 ± 0.82	58.65 ± 1.24	83.39 ± 4.96	90.20 ± 2.61	133.96 ± 1.63	64.06 ± 0.88	176.65 ± 1.05	187.22 ± 1.77

The values represent mean ± standard deviation.
Small letters (i.e., a, b, and c) behind dates indicate significant differences among sampling dates, which were determined using one-way ANOVA with Tukey's *post-hoc* test.

Supplementary Table S4. Comparison of environmental factors among sub-sites on December 23, 2021

Site	pH	Temperature (°C)	Salinity (%)	DO (%)	NH_4^+-N (μg L^{-1})	NO_2^--N (μg L^{-1})	NO_3^--N (μg L^{-1})	PO_4^{3-}-P (μg L^{-1})	SiO_3^{2-}-Si (μg L^{-1})
S9_w	8.30 ± 0.02	10.50 ± 0.09	26.42 ± 0.25	99.10 ± 4.35[b]	5.14 ± 5.60	13.73 ± 2.06	125.14 ± 27.68	7.38 ± 4.16	184.97 ± 77.32
S9_w1	8.28 ± 0.05	10.50 ± 0.00	26.42 ± 0.00	106.10 ± 0.98[a]	2.72 ± 2.34	13.27 ± 0.37	112.66 ± 10.41	4.88 ± 4.31	176.65 ± 71.13
S9_w2	8.33 ± 0.03	10.45 ± 0.03	26.57 ± 0.07	101.00 ± 2.19[b]	1.44 ± 1.31	12.27 ± 1.02	112.70 ± 13.74	4.40 ± 2.44	187.22 ± 67.36

The values represent mean ± standard deviation.
Small letters (i.e., a and b) behind values indicate significant differences among sub-sites.

Supplementary Table S5. Quantification of *Pythium porphyrae* in Haizhou Bay using the present method

Site	Dec 16, 2021	Dec 23, 2021	Jan 6, 2022	Feb 24, 2022
S1	4,214.47 ± 354.50	-	2,737.46 ± 317.00	115.20 ± 40.39
S2	7,161.82 ± 463.45	-	3,339.70 ± 463.18	780.70 ± 161.64
S3	4,067.28 ± 403.20	-	3,201.15 ± 233.85	762.85 ± 110.55
S4	3,186.30 ± 273.46	-	3,150.31 ± 162.14	1,487.20 ± 171.98
S5	2,113.14 ± 262.83	-	6,871.52 ± 687.72	988.58 ± 112.32
S6	802.76 ± 258.60	-	5,514.23 ± 537.06	990.50 ± 174.40
S7	6,689.50 ± 831.01	-	12,074.46 ± 6,675.23	1,316.98 ± 102.24
S8	2,035.23 ± 264.52	-	5,704.34 ± 1941.29	1,160.91 ± 105.90
S9	1,762.86 ± 297.82	-	4,813.46 ± 522.45	513.48 ± 99.64
S10	4,013.15 ± 1,301.50	-	3,359.08 ± 173.48	825.45 ± 349.70
S9_w	-	6,769.85 ± 524.41	-	-
S9_w1	-	31,872.03 ± 2,775.65	-	-
S9_w2	-	33,484.16 ± 6,091.10	-	-
S9_s	-	1,556,911.08 ± 169,880.04	-	-
S9_s1	-	3,318,920.12 ± 537,140.88	-	-
S9_s2	-	5,770,581.64 ± 1,245,946.84	-	-

The values of abundance represent mean ± standard deviation (S1–S10, copies mL^{-1} seawater sample; S9_w–S9_s2, copies g^{-1} sample).

Supplementary Table S6. Quantification of *Pythium chondricola* in Haizhou Bay using the present method

Site	Dec 16, 2021	Dec 23, 2021	Jan 6, 2022	Feb 24, 2022
S1	356.86 ± 46.19	-	338.05 ± 178.69	59.14 ± 16.23
S2	647.75 ± 62.98	-	848.23 ± 109.46	195.12 ± 91.92
S3	468.47 ± 90.85	-	760.64 ± 30.43	103.39 ± 2.99
S4	122.62 ± 14.67	-	316.86 ± 36.47	639.53 ± 64.35
S5	154.12 ± 65.96	-	1,938.79 ± 479.70	124.17 ± 11.89
S6	45.97 ± 29.91	-	1,316.57 ± 88.75	182.68 ± 37.85
S7	150.82 ± 102.37	-	1,644.02 ± 118.75	355.27 ± 69.71
S8	38.20 ± 10.20	-	928.34 ± 89.59	480.08 ± 62.15
S9	71.59 ± 11.03	-	1,029.25 ± 94.57	88.82 ± 10.02
S10	767.04 ± 398.85	-	420.79 ± 64.25	168.89 ± 27.1
S9_w	-	81.34 ± 8.43	-	-
S9_w1	-	640.50 ± 74.25	-	-
S9_w2	-	686.51 ± 168.23	-	-
S9_s	-	21,398.64 ± 3,112.89	-	-
S9_s1	-	58,766.50 ± 12,649.32	-	-
S9_s2	-	123,119.29 ± 35,705.51	-	-

The values of abundance represent mean ± standard deviation (S1–S10, copies mL^{-1} seawater sample; S9_w–S9_s2, copies g^{-1} sample).

Supplementary Table S7. Comparison of pathogen abundance in environmental samples on December 23, 2021

Sample	Groups	ANOVA	Tukey's *post-hoc* test	Unpaired t-test
Seawater	S9_w	****	S9_w vs. S9_w1, ****	
	S9_w1		S9_w vs. S9_w2, ****	
	S9_w2		S9_w1 vs. S9_w2, ns	
Sediment	S9_s		S9_s vs. S9_s1, **	
	S9_s1		S9_s vs. S9_s2, ***	
	S9_s2		S9_s1 vs. S9_s2, *	
Seawater vs. sediment	(S9_w, S9_w1, S9_w2) vs. (S9_s, S9_s1, S9_s2)			****

ns, not significant with p-value > 0.05; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

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