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Quantitative detection of *Vibrio alginolyticus* strain XSBZ14 by a newly developed RT-PCR method

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Abstract

Purpose Coral degradation is a worldwide ecological problem. Bacterial diseases are a great danger to coral health. The pathogenic bacterium *Vibrio alginolyticus* XSBZ14 isolated from diseased coral had been identified as the pathogenic bacterium of *Porites andrewsi* White syndrome (PAWS) in Xisha Archipelago on transmission experiment. To date, the molecular mechanism by which this pathogen causes disease is unknown, and molecular diagnostics for diseases caused by this bacterium have not been developed. In an effort to restore damaged coral ecosystems in the South China Sea, efforts are underway to transplant flat-branch shore corals. There is therefore an urgent need to further develop specific and rapid detection methods for *V. alginolyticus* XSBZ14 in order to prevent this epidemic and ensure the successful implementation of compilation transplants.

Methods At first, a low sequence identity single-copy sequence S2 was selected from the genome by in-house Perl script. Using the designed specific primers, four different types of standard curves were subsequently plotted for the accurate quantification of the strain XSBZ14 in four different samples (DNA, bacterial suspension, coral tissue, seawater). Then, use the strain to infect the *Galaxea fascicularis* and test the strain in the coral culture water during the week.

Results The rapid detection method of pathogenic bacteria by RT-PCR was established. The limit of detection (LOD) of the RT-PCR was 0.88 pg/reaction (0.44 pg/μL) in DNA, 2 CFU/reaction (1000 CFU/mL) in bacterial suspension, 2 CFU/reaction in coral tissue, and 20 CFU/reaction in seawater for the strain XSBZ14, respectively. In addition, according to the detection results of the RT-PCR, the strain XSBZ14 could survive in *Galaxea fascicularis* for a week, and the strain could also be detected from its reared seawater.

Conclusion These results indicated that the RT-PCR detection method of a coral pathogenic strain XSBZ14 was established. The method is a robust tool for the rapid detection and quantification of the coral pathogen, XSBZ14, and is very useful for PAWS epidemiological survey and specific pathogen-free coral transplantation in the South China Sea. And other coral species and their habitats might act as an important reservoir for the strain XSBZ14 and mediated its horizontal transmission in coral reefs.

Keywords *Vibrio alginolyticus*, Coral pathogen, RT-PCR, Single-copy sequence

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Introduction

Coral reefs are the most inherently biodiverse ecosystems in the ocean (Heron et al. 2017), as well as playing a vital role in supporting local communities by way of coastal protection, food production, and tourism (Moberg and Folke 1999; Rosenberg et al. 2007a; Bourne et al. 2009; Wilson et al. 2013). The coral reef ecosystems provide social, economic, and cultural services with an estimated value of over US $1 trillion globally and over half a billion people's economic benefits around the world (Hoegh-Guldberg et al. 2015; Costanza et al. 2014). The health of coral reefs is rapidly deteriorating worldwide due to human disturbances and natural disasters (Xiao et al. 2022). Coral species diversity and cover declined on a massive scale. Coral bleaching, disease outbreaks, and competition brought on by the survival dominance of macroalgae are causing corals to fail to recover naturally from disturbances, and coral reefs are losing their great biodiversity and ecosystem functions (Xiao et al. 2022). According to the survey, the hard coral covered on Caribbean reefs has decreased by an average of 80% in the last 30 years (Gardner et al. 2003; Pollock et al. 2011; Randazzo-Eisemann et al. 2022); Indo-Pacific reefs were severely affected as well, with an estimated coral cover loss of 50% (Bruno and Selig 2007; Johnson et al. 2022); and the coverage of live coral in the South China Sea declined by more than 30% over the past few decades (Qiu et al. 2010; Zhu et al. 2012; Huang et al. 2006; Shi et al. 2012; Schul et al. 2022). In 2020, the bleaching rate was 23.90% overall and topped out at 49.30% in Bei Jiao (Xiao et al. 2022).

In the context of global threats from global warming and ocean acidification, an increasing number of disease outbreaks further endanger the health and survival of corals (Tracy et al. 2019). Most of the identified coral bacterial diseases belong to *Vibrio*. *Vibrio* is the dominant species in marine ecosystem and the main pathogen of coral disease (Xie et al. 2013; Munn 2015; Kemp et al. 2018; Rubio-Portillo et al. 2020). For instance, *Vibrio shilonii* produced toxic peptides that altered the PH of the microenvironment and inhibited photosynthesis in zooxanthellae. And it could penetrate coral tissue, survive in coral cells, and erupt at high temperatures, which was the cause of seasonal bleaching in the Mediterranean. *Vibrio coralliilyticus* was a pathogen of tropical corals that produced a metalloproteinase that caused rapid tissue lysis and death of corals. In addition, *V. alginolyticus*, *Vibrio natriegens*, *Vibrio parahaemolyticus*, and *Vibrio harveyi* all had been reported to cause disease outbreaks in coral (Xie et al. 2013; Munn 2015). Unfortunately, deficiency in effective and convenient detection tools of the causative agents of coral diseases making studies on coral disease etiologies and transmission dynamics had been frustrated for most of the observed diseases (Li et al. 2018). Robust detection tools with high specificity and sensitivity for target pathogens would enable investigations of the circumstances under which microbes that are usually found on corals become pathogenic and the conditions and mechanisms that trigger a switch from commensal or neutral to pathogenic (Pollock et al. 2011). Convenient detection tools would also increase our capacity to establish links between disease symptom and the presence of specific microbial agents, which could improve coral disease classification and diagnosis. These capabilities would be helpful for reef managers to discern the threats that impact the occurrence, prevalence, and severity of diseases, so their sources could be identified and possibly reduced through better management practices (Bruckner 2002). Thus, the development of sensitive, specific, and robust coral disease detection tools should be an alternative priority of coral reef protection (Bourne et al. 2009).

The pathogenic bacterium *V. alginolyticus* XSBZ14 isolated from diseased coral had been identified as the pathogenic bacterium of *Porites andrewsi* White syndrome (PAWS) in Xisha Archipelago on transmission experiment (Xie et al. 2013). To date, the molecular mechanism by which this pathogen causes disease is unknown, and molecular diagnostics for diseases caused by this bacterium have not been developed. In an effort to restore damaged coral ecosystems in the South China Sea, efforts are underway to transplant *Porites andrewsi*. There was an urgent need to further develop specific rapid tests for *V. alginolyticus* XSBZ14 to prevent the spread of this epidemic disease and ensure the successful implementation of coral transplantation.

Since virulence genes related to pathogenesis of *V. alginolyticus* XSBZ14 had not been clearly studied, we chose the sequence of single copy of the genome as the target sequence during the development of diagnostic tools. Single-copy genes refer to only one or a few copies of genes in the genome, most of which are housekeeping genes in organisms (Wang et al. 2006). These genes could be potential targets for coral pathogens detection. In this study, single-copy sequences in the strain XSBZ14 genome were screened by our in-house designed Perl script. Fortunately, the single-copy sequence S2 (GenBank accession number: MH702378) with 94.67% sequence identity for CP013485 (region: 845157 ~ 846788) on NCBI GenBank was selected as a target, and the real-time PCR (RT-PCR) detection method of the strain XSBZ14 based on S2 has been established successfully. The method showed high sensitivity among various samples and specificity among all tested strains.

Materials and methods

Bacterial strains

A total of 73 *Vibrio* strains were used in this study and listed in Table 1. The coral causative agent *V. alginolyticus* XSBZ14, which caused PAWS, was isolated from sick corals. Six *Vibrio* species reference standard strains (*V. alginolyticus*, *V. harveyi*, *V. vulnificus*, *V. natriegens*, *V. fluvialis*, *V. parahaemolyticus*) were obtained from the American Type Culture Collection (ATCC), and ten *Vibrio* strains (seven *Vibrio owensii*, *Vibrio coralliilyticus, Vibrio mediterranei, V. shilonii*) were purchased from Marine Culture Collection of China (MCCC). Fifty-five other *Vibrio* strains were isolated from corals in the Xisha Archipelago, marine rearing systems, seawater, and diseased fish, respectively. They were all identified by the methods reported previously (Xie et al. 2005; Cano-Gomez et al. 2009).

Bacterial culturing and DNA extraction

All of the *Vibrio* strains were grown in 5-mL marine broth 2216E at 30 °C with 180 rpm shaking for 16 h. DNAs were extracted and purified using Bacterial Genomic DNA Extraction Kit Ver.3.0 (TaKaRa, Japan). Extracted DNAs were eluted with 100-μl TE buffer. The concentration and purity of extracted DNAs were quantified using NanoDrop 2000 (Thermo Fisher, USA), and the DNA samples were stored at −20 °C until use.

Target sequences and primer design

Our in-house designed Perl script was used to screen single-copy sequences from the strain XSBZ14 genomic data. Specific single-copy sequences with low sequence identity (percent identity ≤ 98%) on NCBI GenBank were further screened by BLASTn. Specific single-copy sequences were alignment with the sequences of their highest sequence identity on NCBI GenBank by DNAMAN 6.0, respectively. Specific targets were selected according to the alignment results. Primers were designed based on these specific targets. Primer-BLAST was performed to check the specificity of primers (https://www.ncbi.nlm.nih.gov/tools/primer-blast/, parameters: database = nr, organism = bacteria (taxid: 2)). The primers, only can amplify the sequence of the strain XSBZ14 on Primer-BLAST, were synthesized by the Beijing Genomics Institute (BGI) and further verified by PCR.

Specificity of RT-PCR assay

RT-PCR was performed according to ChamQ Universal SYBR qPCR Master Mix (Vazyme, China). The RT-PCR reaction contained 10 μL 2 × ChamQ Universal SYBR qPCR Master Mix, 0.4 μL each primer (10 μM), 2 μL template, and deionized sterile water to a final volume of 20 μL. The thermal program consisted of (i) 5 min at 95 °C; (ii) 40 cycles of 15 s at 95 °C, 1 min at 65 °C; and (iii) a final 7 min at 72 °C. The extracted DNA from each of the *Vibrio* strains and negative control (no template control (NTC)) were used as a template to verify the specificity of RT-PCR. The RT-PCR products were further detected by gel electrophoresis.

Standard curves and limit of detection (LOD)

Four different types of standard curves were constructed to quantify the strain XSBZ14 in four samples (DNA, bacterial suspension, coral tissue, seawater).

The initial concentration of the strain XSBZ14 DNA was quantified by NanoDrop 2000 (Thermo Fisher, USA) and performed tenfold serial dilutions with deionized sterile water. Two microliters of the diluted DNA was used as the template for RT-PCR to construct standard curves and define the LOD of DNA.

The initial concentration of the strain XSBZ14 suspension was quantified by agar plate method (Li et al. 2018) and was performed tenfold dilutions with sterile phosphate-buffered saline (PBS). Two microliters of the diluted bacterial suspension of the strain XSBZ14 cells was used as the template for RT-PCR to construct standard curves and define LOD of the strain XSBZ14 cells.

Tenfold dilutions of the strain XSBZ14 cells were added into 1-g coral tissue (equivalent final number was 10^6 to 0 CFU). The DNA of coral tissue with the strain XSBZ14 was extracted using FastPure Cell/Tissue DNA Isolation Mini Kit (Vazyme, China) and was eluted with 100-μl TE buffer. Two microliters of the DNA were used as the template for RT-PCR to construct standard curves and define the LOD of the strain XSBZ14 in coral tissue.

Tenfold dilutions of the strain XSBZ14 cells were added into 1-L seawater (equivalent final number was 10^7 to 0 CFU). The entire volume of seawater with the strain XSBZ14 was filtered through a Sterivex-GP filter (Millipore), and DNA was extracted using the Water DNA Isolation Kit (Foregene, China) and was eluted with 100-μl TE buffer. Two microliters of the DNA was used as the template for RT-PCR to construct standard curves and defined LOD of the strain XSBZ14 in reared seawater.

Real-time PCR application

Healthy *Galaxea fascicularis* and reared seawater were detected by the above RT-PCR method. Then, healthy *Galaxea fascicularis* were incubated for 24 h in 10-L reared seawater containing the strain XSBZ14 at a density of 10^7 CFU/mL. Incubated corals were transferred to the aquarium tank and maintained the temperature at 29 ± 1 °C. Incubated coral tissue and reared seawater were detected by the RT-PCR method every 24 h for a week. Coral tissue DNA was extracted using FastPure Cell/

Table 1 Tested strains and specific detect of RT-PCR

Number	Strain	Sources	$C_T \pm SD$
1	*V. alginolyticus* XSBZ14	Diseased *P. andrewsi*, Xisha Archipelago	11.35 ± 0.09
2	*V. alginolyticus* ATCC17749	ATCC	NA
3	*V. alginolyticus* HN07014	Reared seawater, Hainan, China	NA
4	*V. alginolyticus* HN07011	Reared seawater, Hainan, China	32.96 ± 1.25
5	*V. alginolyticus* HN08809	Diseased *Epinephelus coioides*, Haikou	NA
6	*V. alginolyticus* HN08304	Reared seawater, Haikou	NA
7	*V. alginolyticus* HN08307	Reared seawater, Xincun Bay in Lingshui City	NA
8	*V. alginolyticus* HN08805	Reared seawater, Hainan, China	NA
9	*V. alginolyticus* HN07005	Seawater, Haikou, Hainan, China	34.35 ± 1.78
10	*V. alginolyticus* XSE381	Healthy Corals, Xisha Archipelago	35.83 ± 2.47
11	*V. alginolyticus* HN08803	Reared seawater, Hainan, China	NA
12	*V. alginolyticus* PBVAY30031	Reared seawater, Hainan, China	NA
13	*V. alginolyticus* HN08203	Reared seawater, Hainan, China	NA
14	*V. alginolyticus* HN07006	Diseased *Lutjanus argentimaculatus*, Hainan, China	NA
15	*V. alginolyticus* HN08811	Diseased *Epinephelus coioides*, Haikou	NA
16	*V. alginolyticus* HN08155	Diseased *Epinephelus coioides*, Xincun Bay in Lingshui City	33.06 ± 0.5
17	*V. alginolyticus* HN07002	Seawater, Haikou, Hainan, China	NA
18	*V. alginolyticus* TG06003	Seawater, Hainan, China	NA
19	*V. alginolyticus* HN08306	Seawater, Hainan, China	NA
20	*V. alginolyticus* HN07009	Seawater, Hainan, China	NA
21	*V. alginolyticus* HN08813	Diseased *Epinephelus coioides*, Haikou	NA
22	*V. alginolyticus* PBVAY39118	Seawater, Hainan, China	NA
23	*V. alginolyticus* HN08335	Diseased *Epinephelus coioides*, Xincun Bay in Lingshui	31.96 ± 1.12
24	*V. alginolyticus* HN08303	Reared seawater, Hainan, China	NA
25	*V. alginolyticus* HN08801	Reared seawater, Hainan, China	NA
26	*V. alginolyticus* HN08201	Reared seawater, Hainan, China	34 ± 1.8
27	*V. alginolyticus* E167	Seawater, Hainan, China	NA
28	*V. alginolyticus* HN08305	Reared seawater, Hainan, China	NA
29	*V. alginolyticus* E001	Healthy Corals, Xisha	NA
30	*V. alginolyticus* HN08156	Pearl River Fisheries Research Institute, Guangzhou, Guangdong China	31.39 ± 0.03
31	*V. alginolyticus* HN07010	Reared seawater, Hainan, China	NA
32	*V. alginolyticus* HN13001	Diseased *Epinephelus coioides*, Haikou	36.37 ± 1.23
33	*V. alginolyticus* PBVAY40119	Diseased *Epinephelus coioides*, Haikou	NA
34	*V. alginolyticus* HN08202	Reared seawater, Hainan, China	NA
35	*V. alginolyticus* PBVAY41120	Reared seawater, Hainan, China	NA
36	*V. alginolyticus* PBVAY38117	Reared seawater, Hainan, China	NA
37	*V. alginolyticus* HN08806	Reared seawater, Hainan, China	NA
38	*V. alginolyticus* PBVAY42121	Reared seawater, Hainan, China	NA
39	*V. alginolyticus PBVAY12023*	Reared seawater, Hainan, China	NA
40	*V. harveyi* ATCC14126	ATCC	NA
41	*V. harveyi* WC13D121	Diseased *Lutjanus argentimaculatus*, Wenchang	NA
42	*V. harveyi* HNH11011	Diseased *Epinephelus coioides*, Haikou	NA
43	*V. harveyi* HNH11001	Diseased *Lutjanus sanguineus*, Haikou	NA
44	*V. harveyi* WC13DH21	Diseased *Lutjanus argentimaculatus*, Wenchang	NA
45	*V. harveyi* WC13H252	Diseased *Lutjanus argentimaculatus*, Wenchang	NA
46	*V. harveyi* HNH11009	Diseased *Lutjanus sanguineus*, Haikou	NA
47	*V. harveyi* HNH11013	Diseased *Lutjanus erythropterus*, Wenchang	NA
48	*V. harveyi* WC13DH52	Diseased *Lutjanus argentimaculatus*, Wenchang	34.55 ± 0.14
49	*V. harveyi* NS131241	Diseased *Epinephelus coioides*, Sanya	NA

Table 1 (continued)

Number	Strain	Sources	$C_T \pm SD$
50	*V. harveyi* NS131751	Diseased *Epinephelus coioides*, Sanya	NA
51	*V. harveyi PBVH78461*	Diseased *Epinephelus coioides*, Sanya	NA
52	*V. harveyi* NS131651	Diseased *Epinephelus coioides*, Sanya	NA
53	*V. harveyi* NS131451	Diseased *Epinephelus coioides*, *Sanya*	31.67 ± 0.35
54	*V. harveyi* NS131631	Diseased *Epinephelus coioides*, Sanya	NA
55	*V. harveyi* NS131632	Diseased *Epinephelus coioides*, Sanya	NA
56	*Vibrio owensii* XSBZ03	Diseased *P. andrewsi*, Xisha Archipelago	37.34 ± 3.22
57	*V. owensii* DSM23055	MCCC	NA
58	*V. owensii* MCCC1A07652	MCCC	NA
59	*V. owensii* MCCC1A06874	MCCC	NA
60	*V. owensii* MCCC1A10877	MCCC	NA
61	*V. owensii* MCCC1A06599	MCCC	NA
62	*V. owensii* MCCC1A07291	MCCC	NA
63	*V. owensii* MCCC1A06614	MCCC	NA
64	*V. vulnificus* ATCC 27562	ATCC	NA
65	*Vibrio natriegens* ATCC 33788	ATCC	NA
66	*Vibrio fluvialis* ATCC 33810	ATCC	NA
67	*V. parahaemolyticus* ATCC17802	ATCC	NA
68	*V. parahaemolyticus* PBVPY07150	Seawater, Hainan, China	NA
69	*V. parahaemolyticus* PBVPY06106	Healthy corals, Xisha Archipelago	38.11 ± 1.65
70	*Vibrio rotiferianus* HN076	Healthy corals, Xisha Archipelago	NA
71	*V. coralliilyticus* CAIM616	MCCC	32.88 ± 0.82
72	*Vibrio mediterranei* LMG11258	MCCC	NA
73	*V. shilonii* MCCC1A13957	MCCC	NA
74	Negative control		NA

NA No amplification

Tissue DNA Isolation Mini Kit (Vazyme, China). Reared seawater sample DNA was extracted using Water DNA Isolation Kit (Foregene, China).

Results

Design and verification of the strain XSBZ14-specific primers

One-hundred thirty-one single-copy sequences were screened from the strain XSBZ14 genomic data by our in-house designed Perl script. Five specific sequences, showing low similarities for their homogenous sequences, S2 (94.67%), S4 (98.03%), S5 (98.09%), S6 (97.74%), and S7 (97.54%), were selected as potential target sequence from all of the 131 single-copy sequences by BlASTn (Table 2). Nineteen pair primers were designed based on S2 ($n=3$), S4 ($n=4$), S5 ($n=5$), S6 ($n=4$), and S7 ($n=3$) (Table S1). Only the primers Z14F3/Z14R3 (Table 3) produced positive amplification for the strain XSBZ14 but negative for other tested strains (Figs. S1~S5).

RT-PCR based on primers Z14F3/Z14R3 was carried out among the 73 *Vibrio* strains shown in Table 1.

Table 2 Target sequences and the highest similarity sequences on NCBI GenBank

Target sequence	The highest similarity sequences	Percent identity (%)
S2 (MH702378)	*Vibrio alginolyticus* strain ATCC 33787 (CP013485.1, 845157 to 846788)	94.67
S4 (MK868053)	*Vibrio alginolyticus* strain ATCC 33787 (CP013485.1, 1494698 to 1495408)	98.03
S5 (MK868054)	*Vibrio alginolyticus* strain J207 (CP014040.1, 1115488 to 1116480)	98.09
S6 (MK868055)	*Vibrio jasicida* 090810c (CP025793.1, 2197815 to 2198921)	97.74
S7 (MK868056)	*Vibrio antiquarius* strain EX25 (CP001806.1, 1365137 to 1365868)	97.54

Table 3 RT-PCR primers

Sequence	Primers name	Primer sequences (5′ → 3′)	Products size (bp)
S2	Z14F3	GCGCACTCAAAAAACCTATCAA	244
	Z14R3	ATGCCCAAACAGTTCAGACTC	

The results showed that the cycle threshold (C_T) values were 11.35 ± 0.09 with a single peak melting curve for the strain XSBZ14 DNA, while the C_T values were more than 31 cycles with the bimodal or multimodal melting curves for the other strains and negative control. The electrophoresis gel results of RT-PCR products also showed that only the strain XSBZ14 produced positive amplification. According to the DNA sequencing result, the sequence of the strain XSBZ14 amplified product was consistent with the target segment (244 bp).

Standard curves and LOD of RT-PCR

The initial concentration of the strain XSBZ14 DNA and bacterial suspension is 44 ng/μL and 10^7 CFU/mL, respectively.

Four different types of standard curves could be seen in Fig. 1. The first one showed a good linear correlation between C_T value and the concentration of the strain XSBZ14 DNA, with a correlation coefficient (R^2) of 0.999, a slope of − 3.56, and an efficiency of 101%. The following formula: $y = -3.56\log_{10}x + 17.42$ was

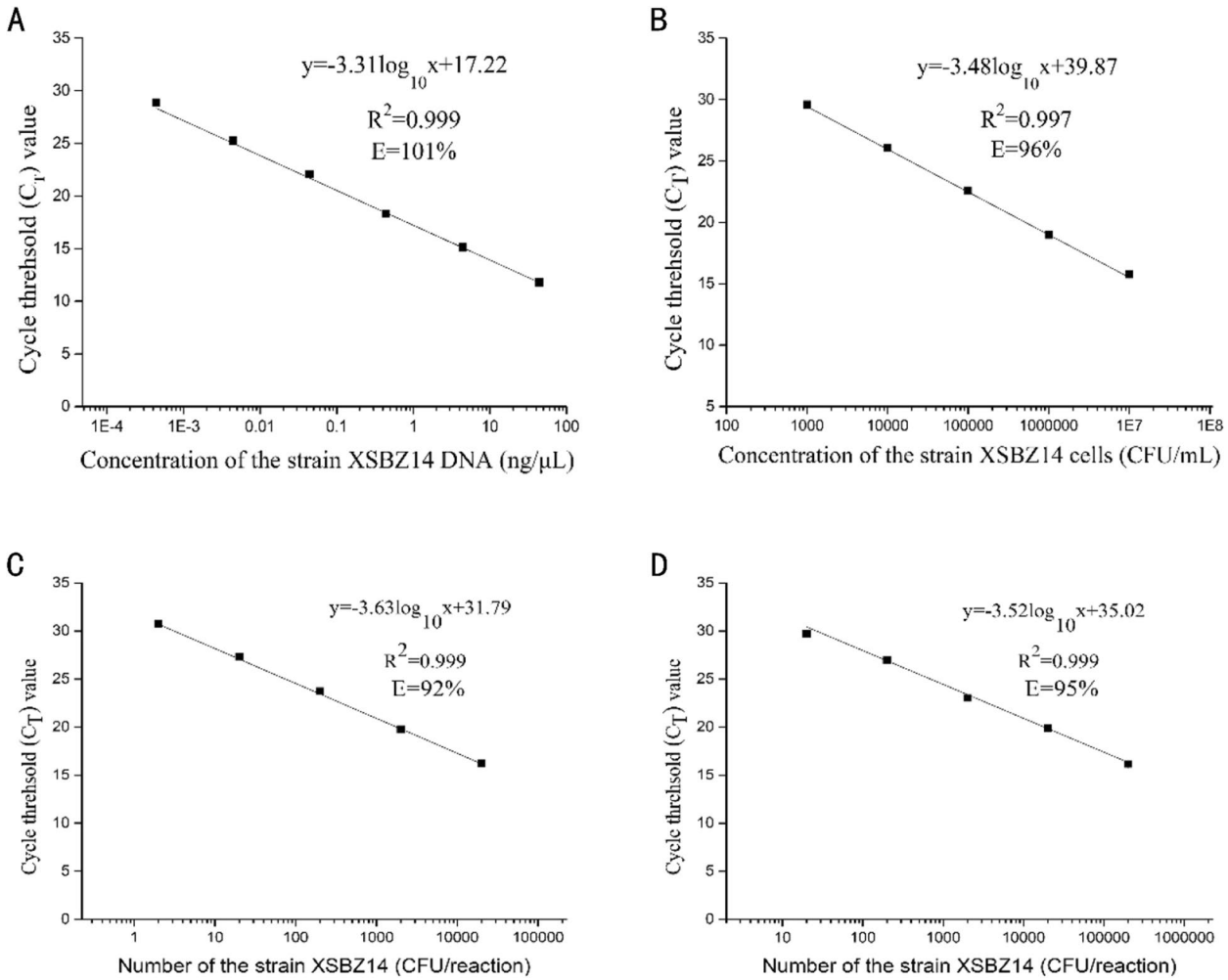

Fig. 1 Standard curves delineating cycle threshold (C_T) values of fluorescence for indicators of pathogen presence. **A** The concentration of XSBZ14 DNA. **B** Concentration of XSBZ14 cells. **C** The number of XSBZ14 cells in coral tissue. **D** The number of XSBZ14 cells in reared seawater

achieved to quantify the concentration of the strain XSBZ14 DNA. The LOD for the strain XSBZ14 DNA was about 0.88 pg/reaction (0.44 pg/μL) (Fig. 1A).

The second standard curve showed a good linear correlation between C_T value and the concentration of the strain XSBZ14 cells, with a correlation coefficient (R^2) of 0.997, a slope of − 3.48, and an efficiency of 96%. The following formula: $y = -3.48\log_{10}x + 39.87$ was achieved to quantify the concentration of the strain XSBZ14 cells. The LOD for the strain XSBZ14 cells was about 2 cells/reaction (10^3 CFU/mL) (Fig. 1B).

The third standard curve showed a good linear correlation between C_T value and the number of the strain XSBZ14 cells in sampled coral tissue, with a correlation coefficient (R^2) of 0.999, a slope of −3.63, and an efficiency of 92%. The following formula: $y = -3.63\log_{10}x + 37.96$ was achieved to quantify the number of the strain XSBZ14 cells in sampled coral tissue. The LOD for the strain XSBZ14 cells in sampled coral tissue was about 2 CFU/ reaction (Fig. 1C).

The fourth standard curve showed a good linear correlation between C_T value and the number of the strain XSBZ14 cells in sampled seawater, with a correlation coefficient (R^2) of 0.999, a slope of −3.52, and an efficiency of 95%. The following formula: $y = -3.48\log_{10}x + 37.96$ was achieved to quantify the number of the strain XSBZ14 cells in sampled seawater. The LOD for the strain XSBZ14 cells in sampled seawater was about 20 CFU/reaction (Fig. 1D).

RT-PCR application

The number of the strain XSBZ14 cells in coral tissues and reared seawater was quantified in different time series according to standard curves of Fig. 2C and D, respectively. The detection results could be seen in Table 4. The strain XSBZ14 had not been detected in healthy coral and natural seawater. The concentration of the strain XSBZ14 in coral tissue could be as high as 3×10^5 CFU/g after immersion. The concentration of the strain XSBZ14 in coral and seawater showed an overall downward trend in the post immersion week. However, the concentration of the strain XSBZ14 was stable at about 3×10^3 CFU/g from the third day to the fifth day in the coral tissue. Only the first day after transferring in reared system, the strain XSBZ14 could be detected in reared seawater.

Discussion

Coral disease outbreaks had become a new strain on coral conservation (Tracy et al. 2019; Xiao et al. 2022; Randazzo-Eisemann et al. 2022; Schul et al. 2022). The huge diversity and variability of coral microbial communities made it difficult to distinguish pathogens from "background noise." Although sequencing technology had greatly improved our understanding of coral diseases (Modolon et al. 2020; Schul et al. 2022), the pathogens and pathogenic mechanisms of most coral diseases were still unknown. Therefore, accurate detection of pathogenic bacteria from background noise was conducive to the study of coral disease etiology and transmission dynamics.

In molecular detection technology, the selection of nucleotide target sequence was particularly important. The target sequence must be able to be inherited stably in the target strain with high specificity and could be distinguished from nontarget sequence. Among them, ribosome (16S, 18S, 23S, ITS) genes were the most commonly used target sequences for molecular detection. Li constructed a common PCR detection method using IGS sequence of coral pathogen XSBZ03 (Li et al. 2018). In the meantime, phylogenetic marker genes, *danJ*, *rpoD*, *luxS*, and *pyrH*, had also been used as target sequences to construct specific detection methods for coral pathogens (Pollock et al. 2010; Joyner et al. 2014; Chimetto Tonon et al. 2017). In addition, virulence genes directly related to pathogenicity had also been used as target genes for molecular detection. For example, gene *vcpA* encoding the zinc metalloproteinase of *V. coralliilyticus* and the P-toxin gene of *V. shilonii* had been used as specific molecular targets (Banin et al. 2001; Sussman et al. 2009; Pollock et al. 2010b; Ushijima et al. 2020).

However, the 16S rDNA gene and genomic phylogenetic marker genes are often unable to accurately distinguish between the target strains and the closely related strains with low genetic differentiation. Meanwhile, the pathogenic mechanism and virulence genes of XSBZ14 were unclear. Therefore, based on the whole genome sequence of XSBZ14, we used bioinformatics technology to screen out highly specific target sequences as potential molecular targets. Using in-house designed Perl script is an effective attempt to screen potential target sequence for the detection of coral pathogen strain. In this study, 131 single-copy sequences in the strain XSBZ14 genome were obtained by using our in-house designed Perl script. We found that the single-copy sequence S2 which has 94.67% sequence identity with the most similar sequence on GenBank was a useful target for establishing the RT-PCR detection method of the strain XSBZ14. This result means that an effective detection method for a coral bacteria pathogen might be developed if a single-copy sequence which has ≤94.67% sequence identity with the most similar sequence on GenBank could be obtained.

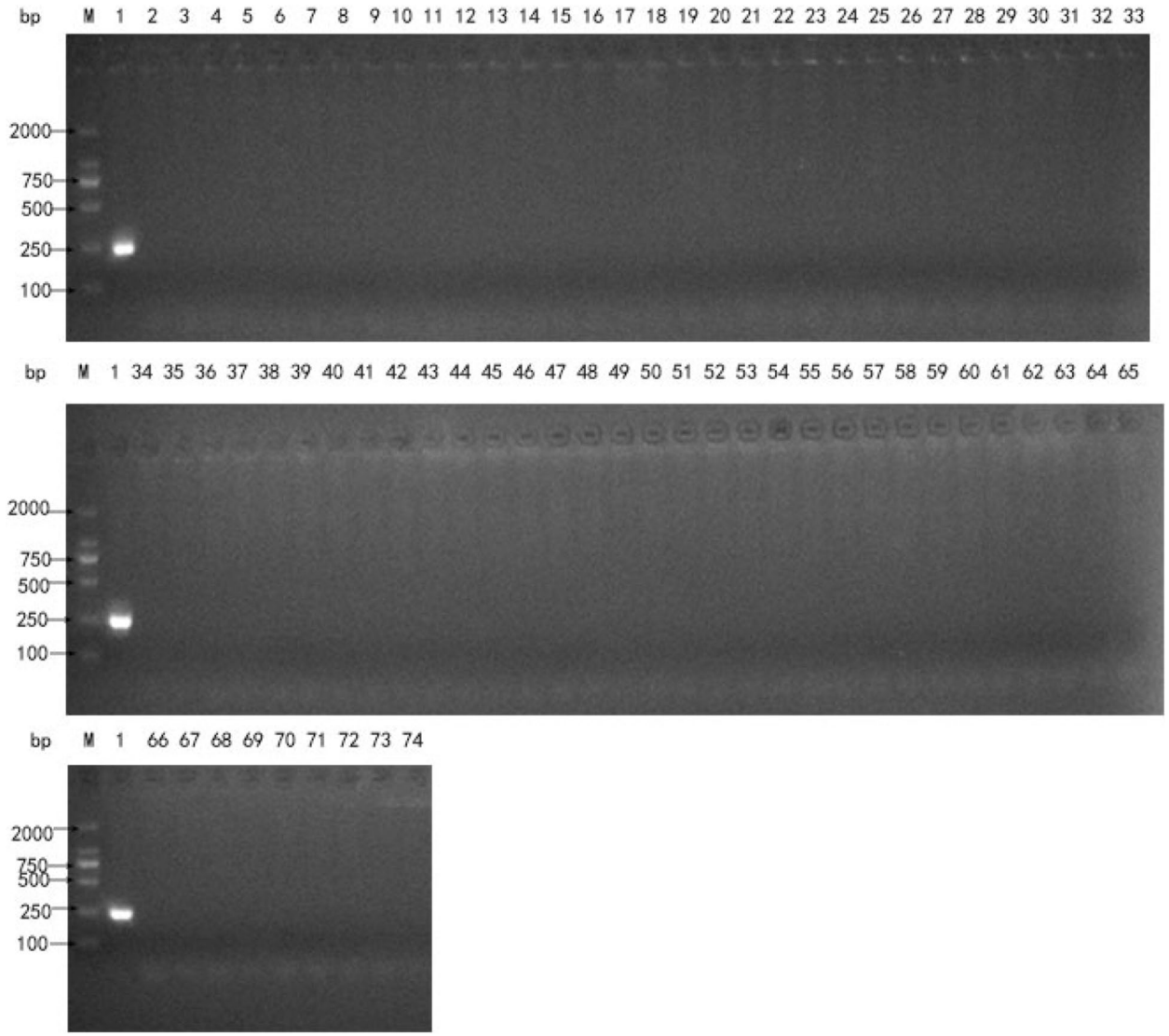

Fig. 2 The gel electrophoresis results of RT-PCR products. Lane M, DL2000 DNA markers; lane 1, XSBZ14; lanes 2–73 could be seen in Table 1; lane 74, negative control

Table 4 The detection of the strain XSBZ14 in coral tissue and reared seawater

Sampling time of incubated coral (day)	Sampling quality of incubated coral (g)	The concentration of the strain XSBZ14 in coral tissue (CFU/g)	Sampling volume of reared seawater (L)	The concentration of the strain XSBZ14 in reared seawater (CFU/L)
0	0.45	3.0×105	1	-
1	0.35	2.8×104	1	5.6×103
2	0.65	1.2×104	1	-
3	0.45	3.0×103	1	-
4	0.50	3.0×103	1	-
5	0.45	3.0×103	1	-
6	0.63	1.4×103	1	-
7	0.40	3.2×102	1	

"-," ND

Fortunately, the RT-PCR detection tool was successfully developed based on a selected single-copy sequence S2 in this study. According to the four plotted standard curves in which R^2 values were 0.999, 0.997, 0.999, and 0.999, strong linear-negative correlations were produced between the C_T values and the concentrations of the extracted DNA, the concentration of the cells in bacteria suspension, the cells in the sampled coral tissue, and the cells in the sampled seawater, respectively. These results had shown better linear relationships than those of the developed method described by Pollock et al. (2010) for detecting *V. coralliilyticus*, in which the R^2 values were 0.998, 0.953, 0.97, and 0.98 for the concentration of DNA and cells, the number of *V. coralliilyticus* in seeded corals and seawater. Simultaneously, the RT-PCR showed a higher sensitivity (10^3 CFU/mL) than that (10^4 CFU/mL) of the detection method developed by Pollock for the suspended cells. For other samples, the sensitivity of the RT-PCR was superior to or equivalent to the reported detection methods of coral pathogens (Pollock et al. 2010; Chimetto Tonon et al. 2017). The only fly in the ointment is the virulence of *Vibrio* depended on activity, and our target sequence did not reflect the activity of XSBZ14.

In the process of using S2 for molecular detection tools, the strain XSBZ14 could be detected from *Galaxea fascicularis* by the RT-PCR with a downward trend in a week. Moreover, the strain XSBZ14 could survive in the coral reared seawater in terms with the detection results. However, the inoculation of the strain XSBZ14 in healthy colonies of *Galaxea fascicularis* did not induced its white syndrome. These results indicated that the strain XSBZ14 might be not the causative agent for *Galaxea fascicularis* but could inhabit in the coral ecosystem, which might exist the variable microbial communities between *Galaxea fascicularis* and *P. andrewsi* (Reshef et al. 2006). Thus, other coral species and their habitats might act as an important reservoir for the strain XSBZ14 and mediated its horizontal transmission in coral reefs.

In conclusion, this paper developed a rapid and sensitive RT-PCR molecular detection method for *V. alginolyticus* XSBZ14 based on S2 single-copy fragment as the target sequence to prevent the outbreak of PAWS in the South China Sea.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13213-023-01726-7.

Additional file 1: Table S1. Primer information. **Figure S1.** Screening of specific primer based on S2. (A). Amplification of primers Z14F1/Z14R1. (B). Amplification of primers Z14F2/Z14R2. (C). Amplification of primers Z14F3/Z14R3. Lane M: DL1000 DNA markers; Lane 1: XSBZ14; Lane 2-32 could be seen in Table 1. **Figure S2.** Screening of specific primer based on S4. (A). Amplification of primers Z14F4/Z14R4. (B). Amplification of primers Z14F5/Z14R5. (C). Amplification of primers Z14F6/Z14R6. (D). Amplification of primers Z14F7/Z14R7. Lane M: DL1000 DNA markers; Lane 1: XSBZ14; Lane 2-32 could be seen in Table 1. **Figure S3.** Screening of specific primer based on S5. (A). Amplification of primers Z14F8/Z14R8. (B). Amplification of primers Z14F9/Z14R9. (C). Amplification of primers Z14F10/Z14R10. (D). Amplification of primers Z14F11/Z14R11. (E). Amplification of primers Z14F12/Z14R12. Lane M: DL1000 DNA markers; Lane 1: XSBZ14; Lane 2-32 could be seen in Table 1. **Figure S4.** Screening of specific primer based on S6. (A). Amplification of primers Z14F13/Z14R13. (B). Amplification of primers Z14F14/Z14R14. (C). Amplification of primers Z14F15/Z14R15. (D). Amplification of primers Z14F16/Z14R16. Lane M: DL1000 DNA markers; Lane 1: XSBZ14; Lane 2-32 could be seen in Table 1. **Figure S5.** Screening of specific primer based on S7. (A). Amplification of primers Z14F17/Z14R17. (B). Amplification of primers Z14F18/Z14R18. (C). Amplification of primers Z14F19/Z14R19. Lane M: DL1000 DNA markers; Lane 1: XSBZ14; Lane 2-32 could be seen in Table 1.

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Authors' contributions
NZ, methodology and writing — review and editing. SY, investigation, methodology, and writing — review and editing. XZ, writing — review and editing. HL, methodology and supervision. YF, supervision. ZX, conceptualization, investigation, and writing — review and editing.

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Availability of data and materials
All data generated or analyzed during this study are included in this article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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