分类号_____ 密级_____

U D C_____ 编号 56686

厦 门 大 学

博 士 后 研 究 工 作 报 告

东海典型有害赤潮藻塔玛亚历山大藻杀藻细菌的研究

及南海可培养细菌多样性分析及几丁质降解菌研究

博士后姓名：**苏建强**

工作完成日期：2009.3

报告提交日期：2009.4

厦门大学

2009 年 4 月

东海典型有害赤潮藻塔玛亚历山大藻杀藻细菌的研究

及南海可培养细菌多样性分析及几丁质降解菌研究

Algicidal bacteria against *Alexandrium tamrense*

Study of culturable bacteria diversity and chitin degrading bacteria in South China Sea (SCS)

博　士　后　姓　名：**苏建强**

流动站（一级学科）名称：**环境科学与工程**

专　业（二级学科）名称：**环境微生物学**

研究工作起始时间：2007.1.24

研究工作期满时间：2009.1.24

厦 门 大 学

2009　年　4　月

厦门大学博士后研究工作报告著作权使用声明

本人完全了解厦门大学有关保留、使用博士后研究工作报告的规定。厦门大学有权保留并向国家主管部门或其指定机构送交该报告的纸质版和电子版，有权将该报告用于非赢利目的的少量复制并允许该报告进入学校图书馆被查阅，有权将该报告的内容编入有关数据库进行检索，有权将博士后研究工作报告的标题和摘要汇编出版。保密的博士后研究工作报告在解密后适用本规定。

本研究报告属于：　　1、保密（　），　2、不保密（　）

纸本在　　年解密后适用本授权书；

电子版在　　年解密后适用本授权书。

（请在以上相应括号内打“√”）

作者签名：　　日期：　　年　月　日

导师签名:　　日期：　　年　月　日

目录

摘 要

本论文分离并鉴定了 9 株塔玛亚历山大藻杀藻细菌，研究了其杀藻方式，构建了其中 5 株杀藻细菌的基因组文库；从南海水样和沉积物样品中进行了细菌的分离和鉴定，并对其中两株几丁质降解菌的特性进行了研究；获得主要结果如下：

从东海赤潮高发区分离并筛选到 9 株塔玛亚历山大藻杀藻菌，16S rDNA 序列分析表明它们分别属于假交替单胞菌属（SP31、SP44）、交替单胞菌属（DH12、DH46）、*Idiomarina* 属（SP96）、弧菌属（DH47、DH51）、盐单胞菌属（DH74、DH77）。对它们的杀藻方式研究表明，这些杀藻细菌都是通过间接方式杀藻，即通过产生胞外杀藻活性物质来杀死藻细胞，直接加入细菌细胞并不产生杀藻活性。大多数这些杀藻细菌产生的杀藻物质具有较高的热稳定性。

分别提取了 5 株杀藻细菌（DH46、DH51、DH77、SP48、SP96）的基因组 DNA，采用 pUC19 质粒作为载体，分别构建了这五株杀藻细菌的基因组文库，文库平均插入片段大约 3-7 kb，克隆子约 5-7 $\times 10^3$ 个，覆盖率达到 99%。

从南海水样和沉积物中分离到细菌 169 株，RFLP 分析得到 55 个谱型，经测序后结果表明所分离到得细菌中 Proteobacteria 占据优势（78.2%），其中 γ-Proteobacteria 占 63.6%，α-Proteobacteria 占 14.5%；Firmicutes 占 21.8%。这些细菌分属于 9 科，15 属，35 种。其中菌株 SCSWE24 与模式菌株同源性最高仅 92%，可能是一株新种。

从南海海域水样和沉积物中分离并筛选到两株几丁质降解菌，分别为 SCSS04 和 SCSWE13。16S rDNA 序列分析结果表明，菌株 SCSS04 属于芽孢杆菌属，菌株 SCSWE13 属于弧菌属。两株菌几丁质酶的产生均需要几丁质的诱导，并在富营养培养基中产酶量较高。菌株 SCSS04 在培养第 9 天产酶量达到最高，所产生的几丁质酶最适反应温度为 40~50℃。菌株 SCSWE13 属于低温酶，在培养第 7 天产酶量达到最高，所产生的几丁质酶最适反应温度为 20~28℃。

关键词：

杀藻细菌，塔玛亚历山大藻，有害藻华，南海，细菌多样性，几丁质降解菌

Abstract

As part of efforts to enhance the strategies employed to manage and mitigate algal blooms and their adverse effects, algicidal bacteria have shown promise as potential suppressors of these events. Nine strains of bacteria algicidal against the toxic dinoflagellate, *Alexandrium tamarense*, were isolated from the Donghai Sea area, China. Sequence analysis of 16S rDNA showed that all the algicidal bacteria belonged to the γ-proteobacteria subclass and the genera *Pseudoalteromonas* (strain SP31 and SP44), *Alteromonas* (strain DH12 and DH46), *Idiomarina* (strain SP96), *Vibrio* (strain DH47 and DH51) and *Halomonas* (strain DH74 and DH77). To assess the algicidal mode of these algicidal bacteria, bacterial cells and the filtrate from bacterial cultures were inoculated into *A. tamarense* cultures, and fluorescein diacetate vital stain was applied to monitor the growth of the algal cells. The results showed that all the algicidal bacteria exhibited algicidal activity through an indirect attack since algicidal activity was only detected in cell free supernatants but not the bacterial cells. This is the first report of bacteria from the genus *Idiomarina* showing algicidal activity to the toxic dinoflagellate *A. tamarense* and these findings would increase our knowledge of bacterial-algal interactions and the role of bacteria during the population dynamics of HABs.

Genome libraries of five strains of algicidal bacteria (DH46、DH51、DH77、SP48、SP96) were constructed using plasmid pUC-19 as vetor. The prepared genomic libraries had average insert sizes of 3-7 kb and contains 5-7 $\times 10^3$ clones respectively, and the coverage rate were more than 99%.

169 strains of marine bacteria were isolated from water sample and sediments collected from South China Sea (SCS). RFLP analysis of bacterial 16S rDNA revealed 55 patterns. Sequence analysis of 16S rDNA from each RFLP pattern show that the isolates were phylogenetically identified as belonging to the Proteobacteria (78.2%) and Firmicutes (21.8%).

2 strains of chitin-degrading bacteria was screened from the water sample and sediment of South China Sea, namely SCSS04 and SCSWE13. Sequence analysis of 16S rDNA showed that strain SCSS04 was belong to genus *Bacillus* and strain SCSWE13 to genus *Vibrio*. Presence of chitin was required for the production of chitinase, and high level of chinase activity occured in eutrophic medium. The chitinase producted by strain

SCSS04 was most active at 40-50℃ and peaked at 9th day. The chitinase producted by strain SCSWE13 belonged to cold active enzyme as it was most active at 20-28℃, and peaked at 7th day in batch cultivation.

Key words:

Algicidal bacteria, Alexandrium tamarense, Harmful algal blooms, South China Sea, Bacterial diveristy, Chitin-degrading bacteria

第一章 塔玛亚历山大藻杀藻细菌的研究

Marine bacteria antagonistic to the harmful algal bloom (HAB) causing alga, *Alexandrium tamarense* (Dinophyceae)

1 Introduction

Harmful algal blooms (HABs) are often linked to significant economic losses through massive fish kills, shellfish harvest closures, and the potential threat to humans of shellfish poisonings. To manage and mitigate the adverse impact of HABs, various strategies have been applied in controlling their outbreak and persistence, involving treatment with chemical agents such as copper sulfate[1], flocculation of microalgae with clay[2] and other physical techniques.

Although effective in controlling blooms, chemical and physical approaches are considered to be potentially dangerous, since chemical agents could cause serious secondary pollution, and they could indiscriminately kill multiple organisms in the aquatic ecosystem, which may alter marine food webs and eventually impact natural fish communities[3]. Biological agents, including bacteria[4], viruses[5], protozoa[3] and macrophytes[6, 7] are considered as potential suppressors in controlling the outbreak and maintenance of algal blooms.

Bacteria play an important role in nutrient regeneration and energy transformation in aquatic ecosystems[8], therefore, algal-bacterial interactions are of particular interest and they have been considered as potentially important regulators of algal growth and toxin production[9]. Research into the relationships between algae and bacteria have resulted in the isolation of strains of algicidal bacteria which mainly belong to the *Cytophaga/Flavobacterium/Bacteroidetes* (CFB) group or to the γ-proteobacteria group, and to the genera *Cytophaga*, *Saprospira*, *Alteromonas* and *Pseudoalteromonas*. These bacteria show algicidal activity through either direct or indirect attack on the target algal cells[4].

Blooms of toxic species of *Alexandrium*, including *A. tamarense*, are often associated with paralytic shellfish poison poisoning cases[10, 11]. The relationship between *Alexandrium* spp. and bacteria has been a subject of interest for years, including studies on the bacterial flora associated with laboratory maintained strains of *Alexandrium* spp.[12, 13], or *Alexandrium* spp. Blooms[10, 14], and the effect of bacteria on toxin production of

algae[15, 16]. However, there are few investigations concerning bacteria algicidal against *Alexandrium* spp.[17, 18]. We previously reported the action of the algicidal bacterium, *Pseudoalteromonas* sp. SP48 against *A. tamarense*. Bacterial isolate SP48 showed algicidal activity through an indirect attack via the excretion of unknown algicidal compounds[19]. In an effort to manage and mitigate the adverse impacts of *A. tamarense*, several strains of algicidal bacteria were isolated and their mode of algicidal activity were studied. The data obtained in this study will increase the information available concerning algicidal bacteria, and improve our understanding of algal-bacterial interactions.

2 Materials and methods

2.1 *A. tamarense* culture

A non-axenic culture of *A. tamarense* ATGD98-006 (provided by the Algal Culture Collection, Institute of Hydrobiology, Jinan University, Guangzhou, China) has been maintained in our laboratory for a number of years. Bacteria from these cultures were removed by repeated washing, together with lysozyme/SDS and antibiotic treatments and the axenic status was confirmed by the lack of bacteria after further cultivation, direct epifluorescence microscopic examination and PCR-based methods[20]. The axenic *A. tamarense* cultures were maintained in f/2 medium (without silicate)[21] prepared with natural seawater (28 psu) at 20±1°C under a 12:12 h light-dark cycle with an illumination of about 50 μE m^{-2} s^{-1}.

2.2 Screening of algicidal bacteria against *A. tamarense*

Bacteria for screening algicidal activity were isolated from surface water samples of a *Prorocentrum donghaiense* Lu bloom (~10^8 cells L^{-1}), accompanied with *A. tamarense* (~10^4 cells L^{-1}), in the East China Sea during the National Science Foundation of China 973 project MC2003-2 cruise on 19-21 May 2003[19]. For initial screening of algicidal activity, 1 mL bacterial cultures grown in 3 mL 2216E broth (25°C, 180 rpm for 12-24 h) were inoculated in triplicate into 50 mL exponentially growing axenic *A. tamarense* ATGD98-006 cultures and 1 mL of 2216E broth only was added (instead of the bacterial cultures) into the algal cultures as a control. The growth of *A. tamarense* was monitored by measuring the relative fluorescence units (RFUs) of the algal cultures every 2 days (excitation wavelength 450 nm and emission wavelength 680 nm) and also by microscopic observation.

2.3 DNA extraction and 16S rDNA sequencing

Protocols for extraction of bacterial genomic DNA and amplification of 16S rRNA gene are described in Su et al (2007). The amplicons were purified from agarose gel with a GeneClean *Turbo* Kit (Qbiogene) and ligated with pMD19-T vector (TAKARA) following by transformation into *E. coli* DH5α competent cells and the clone with a 1.5 kb insert was sent to be sequenced (Invitrogen Biotechnology Co., Ltd). After sequencing, primer and vector sequences were removed using EditSeq 5.0 of DNASTAR. BLAST search was performed through the NCBI Web site (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine the closest phylogenetic types of bacterial isolates. The sequences were aligned and a phylogenetic tree was constructed using DNAMAN 6.0 software with known algicidal bacterial sequences obtained from GenBank.

2.4 Algicidal mode of the algicidal bacteria

Algicidal isolates grown in 3 mL 2216E broth were inoculated into 25 mL 2216E broth and grown to the stationary phase (25°C at 150 rpm for 12-16 h). Bacterial cells were collected by centrifugation (5000 × g, 15 min), washed twice using sterile f/2 medium and re-suspended in sterile f/2. The supernatants were filtrated through 0.22 μm Millipore membrane filters. To determine the algicidal mode of the algicidal bacteria, 0.5 mL bacterial cultures, filtrates or cell suspensions were inoculated into 50 mL axenic *A. tamarense* cultures. Cultures with the addition of 1 mL 2216E but no bacteria served as a control, and a no addition control was also involved in the experiments. Bacterial strain DH21, which was isolated from the same samples, was used as the bacterial control. For DH21 and 1 mL of bacterial culture, filtrate or cell suspension were added into *A. tamarense* cultures. All treatments and controls were performed in triplicate.

The growth of *A. tamarense* was monitored daily using fluorescein diacetate (FDA, Sigma) vital stain. FDA stain was performed according to[22]. FDA (Sigma) working stock (5 mg mL^{-1} in 100% acetone, preserved at 4 °C in the dark) was added into the samples of *A. tamarense* cultures to a final concentration of 50 μg mL^{-1} following by incubation at room temperature for 3 min. The treated samples were kept in an ice bath and vital cells of *A. tamarense* were measured immediately by counting the green cells under an epifluorescence microscope (Olympus BX41) with blue light excitation.

2.5 Heat stability of algicidal activity

Algicidal strains were grown in 100 mL flasks containing 20 mL 2216E broth at 25°C 150 rpm for 24 h. Bacterial cultures were centrifuged (10000 × g, 10 min) and the 0.22 μm filtered supernatants were kept in a boiled water bath for one hour. 1 mL treated

filtrates were inoculated into 40 mL *A. tamarense* cultures (about 2×10^4 cells mL^{-1}) and the same volume of untreated filtrates was used as control. After inoculation for 1 d, the living algal cells were counted with FDA assay. Algal cell mortality, representing the algicidal activity, was calculated using the following equation:

$$\text{Algal cell motality (\%)} = \frac{N_0 - N_1}{N_0} \times 100\%$$

where N_0 refers to the initial living algal cells concentration, N_1 refers to the concentration of living algal cells after inoculation for 1 d.

3 Results

3.1 Isolation of algicidal bacteria

183 bacterial strains were isolated from surface seawater samples and 23 strains of bacteria displayed negative effects on the growth of *A. tamarense* in the preliminary screening. Over an additional two screening experiments, 10 out of the 23 isolates were confirmed to have algicidal activity, including strain SP48, which has been reported and classified as a member of the genus *Pseudoalteromonas*. The other algicidal isolates were designated as strains DH12, DH46, DH47, DH51, DH74, DH77, SP31, SP44 and SP96.

3.2 Identification of algicidal bacteria

Based on the BLAST results for the partial sequences of 16S rDNA, the algicidal isolates were closely related to the following genera: DH12 and DH46, related to *Alteromonas*; DH47 and DH51, *Vibrio* related species; DH74 and DH77 related to *Halomonas*; SP31 and SP44 related to *Pseudoalteromonas*; SP96, an *Idiomarina* related species (Table 1). A phylogenetic tree was constructed with reported algicidal bacteria including our isolates, which all belonged to the γ-proteobacteria. The phylogenetic tree showed clearly that strain SP96 formed a branch separate from all the other algicidal species (Fig. 1). 16S rDNA sequences of the isolates that were shown to have algicidal activity against *A. tamarense* were submitted to the NCBI GenBank and deposited into the Marine Culture Collection of China (MCCC). GenBank accession no. and MCCC deposit no. are shown in Table 1.

3.3 Algicidal activity

The effect of algicidal bacteria on the growth of *A. tamarense* was tested. Bacterial cells and extracellular substances were separated and inoculated into *A. tamarense* cultures separately to investigate the algicidal mode of our isolates. The results are

graphically illustrated in Fig. 2~5. Addition of 1 mL 2216E showed no effect on the growth of *A. tamarense* compared to the no-addition control. Isolate DH21, which was identified as *Marinobacter* sp. by 16S rDNA sequence analysis (GenBank accession no. FJ404750), did not have any significant effect on the growth of *A. tamarense* despite whether it involved the addition of bacterial culture, filtrate or bacterial cells ($p>0.05$ Fig. 2).

With FDA vital stain, living or dead *A. tamarense* cells could be easily distinguished under epifluorescence microscopy with blue light excitation. The living cells were stained as bright green particles, while other algal cells stayed red or gray were considered as dead cells. No significant difference was observed between the treatments with washed bacterial cell inoculation and controls ($p>0.05$ Fig. 2~5). A varying extent of algicidal activity was observed after bacterial cultures or filtrates were introduced into *A. tamarense* cultures, which may indicate the level of algicidal activity among the different algicidal bacteria.

The algal biomass was decreased after inoculation with bacterial cultures of strains DH12, 46, 47 and 51. However, when filtrates were added into the algal cultures, the algal biomass was first decreased, but some *A. tamarense* cells survived the algicidal activity and grew to measurable concentrations by day 5 (Fig. 2 and 3). The different effect between bacterial cultures and filtrates may be due to the loss of algicidal activity in the filtrate with time, since the treatments involving addition of bacterial cultures, the bacteria could utilize the concomitant nutrients for the production of substances deleterious to *A. tamarense*, thus maintaining the algicidal activity. Both bacterial cultures and filtrates of strains DH74, DH77, SP31, SP44 and SP96 exhibited similar algicidal activity to *A. tamarense*. The algal cells were nearly 100% killed by day 5 (DH74), day 2 (DH77, SP31, SP44) and day 1 (SP96) (Fig. 4 and 5).

The cytolysis of algicidal bacteria on *A. tamarense* cells during the algicidal process were similar to that by *Pseudoalteromonas* sp. SP48 previously described [19]. *A. tamarense* cells were observed with reduced mobility, detached cell walls, disrupted intracellular structure and broken cell walls, and cellular substances were released and decomposed, resulting in the appearance of abundant broken thecae.

These results indicated that all 9 strains of algicidal bacteria displayed negative effect on the growth of *A. tamarense* through indirect attack, since no algicidal activity was observed after inoculation of bacterial cells. Excretion of unknown algicidal compounds

in the filtrates by the bacteria was responsible for the algicidal activity. Heat stability of algicidal activity was investigated by comparing the different impact between heat treated and non-treated bacterial filtrates on the growth of *A. tamarense*. Most of the supernatants retained algicidal ability after 1 h in boiled water bath. However, significant difference of algicidal activity ($P<0.01$) occurred in the cases of strain DH12, DH74, and SP31 (Fig. 6). Algicidal activity was obviously decreased by heat treatment, resulting in the survival of algal cells, especially in SP31, *A. tamarense* cells exhibited similar growth rate to the non addition control during the following monitoring (Data not shown).

4 Discussion

In this study, we reported 9 strains of algicidal bacteria, isolated from the East China Sea, antagonistic to *A. tamarense* and studied their algicidal modes. The 9 isolates all belong to the γ-proteobacteria, two are from the genus *Pseudoalteromonas*, two from the genus *Alteromonas*, two from the genus *Halomonas*, two from the genus *Vibrio* and one from the genus *Idiomarina*, according to the analysis of partial 16S rDNA sequence. This notable diversity is not surprising given the diversity of the known algicidal bacteria, most of which phylogenetically belong to either the CFB or the γ-proteobacteria groups, including several genera[4]. However, in this study, the algicidal isolates against *A. tamarense* were all from the γ-proteobacteria group and family Alteromonadaceae, Vibrionaceae and Halomonadaceae, but not the CFB group.

Alteromonas and *Pseudoalteromonas* of the γ-proteobacteria are prevalent in many marine environments, and that they are the most dominant genera among the known algicidal bacteria[23-28]. Several species of *Vibrio* include clinically important human pathogens. Most disease causing strains are associated with gastroenteritis but can also infect open wounds and cause septicemia. *Vibrio* is also reported with algicidal activity to *Gymnodinium mikimotoi*[29] and other raphidophytes[26]. *Halomonas* is common in aquatic environments and can tolerate (and in some cases require) medium or high concentrations of salt for growth. A bacterium closely related to *Halomonas* was isolated with algal-lytic activity to the brown-tide alga *Aureococcus anophagefferens*[30]. The algicidal activity of the two isolates DH74 and DH77 was the second report that organisms from the genus *Halomonas* had algicidal activity. *Idiomarina* was first reported by Ivanova as a new genus[31]. In this study, it was the first description of a bacterium closely related to *Idiomarina* having algicidal activity.

Concerning the diversity of the bacteria algicidal against *A. tamarense*, questions

arose as to what role they played in the termination of HABs. It was suggested that algicidal bacteria that are harmful to or feed on algae would be an important factor in the termination of algal blooms[32, 33]. The role of bacteria would be more important than nutrient depletion and groundwater nutrient inflow, since bloom formation was accompanied with an increase of bacteria abundance which was maintained at a high level during bloom decline[26]. A conceptual model suggested that algicidal bacteria exist as an component of the ambient microbial flora with target algal cells either absent or in low abundance, respond to the increased algal cells with incremental both absolute and relatively abundance, ultimately enhance the process of bloom termination combined with other factors[32]. On the assumption that these algicidal bacteria are responsible for the termination of HABs, conundrum still remains that is there any congenerous mechanism involved during the algicidal process. However, in this study, the role of bacteria in the succession of algal bloom remained unclear due to the lack of in situ data concerning the population dynamics of both algae and algicidal bacteria.

Although *Prorocentrum donghaiense* was the dominant algal specie during the bloom, none of the 183 bacterial isolates manifested algicidal or inhibitory effect on the growth of *Prorocentrum donghaiense*, even on condition that the bacterial cultures was introduced into the *P. donghaiense* cultures at a final concentration higher than 10^9 cells mL^{-1} (data not shown). Most of the reported algicidal bacteria are algal species-specific with no clear pattern[4] and most of the target algae are bloom forming species including flagellates and diatoms due to the expectations of employing algicidal bacteria in the biological control of HABs[26, 34-36]. The lack of *P. donghaiense* antagonistic bacteria may due to the insufficiency in bacteria isolation and screening methods, resulting in the loss of opportunity to discover the bacteria against *P. donghaiense* which may be present in the sea. The associated bacteria community could be another reason that take the responsibility for the resistance to bacteria[33, 37]. If not so, one might hypothesized that, besides the other factors, mortality of *A. tamarense* caused by the presence of specific algicidal bacteria could be one of the factors that prompted *P. donghaiense* becoming the dominant species in the area, considering that *A. tamarense* exerted an inhibitory effect on the growth of *P. donghaiense* under controlled laboratory conditions[38]. This supposition still needs evident proof from field investigation data.

Bacteria that inhibit the growth of algae take effect through direct[33, 37, 39] or indirect attack [17]. The former needs a direct cell to cell contact, while indirect attacks are thought

to be chemically mediated. Our results which showed that the growth of *A. tamarense* was not affected by bacterial cells but by cell free filtrate indicated that the algicidal isolates exhibited algicidal activity through indirect attack and bacteria attachment is not obligate for the mortality of algal cells to occur. Unknown freely diffusible algicidal substance(s) released by the algicidal bacteria could be by-products of the metabolism of media components and were responsible for the death of *A. tamarense*. These algicides would be ecto-proteases[24], peptides[40], biosurfactants[41, 42] or antibiotic-like substances[43]. Most of the filtrates retain algicidal activity after heat treatment at 100 °C for 1 h, which exclude enzymatic mechanisms in these cases. Nonetheless, further illustrations of algicidal mechanisms needs isolation and identification of algicidal compounds.

An interesting phenomenon during our study was observed with a small number of cyst-like cells appearing later in the time course of the experiments. These cyst-like cells appeared only in the cultures treated with algicidal bacteria. It was suggested that the formation of these cyst-like cells was a possible mechanism by which the algal cells avoided attack by algicidal bacteria or substances[33]. These putative cysts was not always found but only observed in *Pseudoalteromonas* mediated algicidal process in our study. Viability of these cells needs to be conformed before drawing a conclusion.

To conclude, our results showed high diversity of algicidal bacteria against *A. tamarense*, providing possible choices for controlling HABs by microbial strategies. Mechanistic researches, including isolation and identification of responsible algicidal substances and illustration of biochemical process, are required for better understanding of the algicidal courses. The role of algicidal bacteria played in phytoplankton death during harmful algal blooms demands explicit evidence from natural conditions.

Table 1. Taxa of algicidal bacterial isolates

Isolates	GenBank accession no.	Group	Genus	MCCC* no.
DH12	FJ404749	γ-proteobacteria	*Alteromonas*	1F01101
DH46	FJ404751	γ-proteobacteria	*Alteromonas*	1F01103
DH47	FJ404752	γ-proteobacteria	*Vibrio*	1F01104
DH51	FJ404753	γ-proteobacteria	*Vibrio*	1F01105
DH74	FJ404754	γ-proteobacteria	*Halomonas*	1F01106
DH77	FJ404755	γ-proteobacteria	*Halomonas*	1F01107
SP31	FJ404756	γ-proteobacteria	*Pseudoalteromonas*	1F01108
SP44	FJ404757	γ-proteobacteria	*Pseudoalteromonas*	1F01109
SP96	FJ404759	γ-proteobacteria	*Idiomarina*	1F01111

* Marine Culture Collection of China