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ORIGINAL PAPER

Phylogenetic diversity and characterization of 2-haloacid degrading bacteria from the marine sponge *Hymeniacidon perlevis*

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Abstract A total of 139 2-haloacid degrading bacteria strains were isolated from the marine sponge *Hymeniacidon perlevis* using a modified enrichment medium and a pH indicator method. After screening on indicator agar and 2-chloropropionic acid (2-CPA) liquid medium, 11 isolates with high degrading activities were characterized and initially identified. Seven of the 11 isolates were able to degrade 2-CPA at 8% salt, and four isolates (DEH 66, DEH 99, DEH125 and DEH138) degraded 2-CPA at 15% salt. Eight of the 11 isolates utilized all four types of organohalogen compounds used in this study. The DEH99 and DEH138 isolates exhibited the best enantioselectivity towards (S)-2-chloropropionic acid (S-CPA) and (R)-2-chloropropionic acid (R-CPA), respectively. The dehalogenase activities of DEH84 against racemic CPA, DEH99 against S-CPA, DEH138 against R-CPA and DEH130 against racemic CPA were 0.16U/mg, 0.06U/mg, 0.12U/mg and 0.19U/mg, respectively. Based on 16S rRNA sequence analysis, the 11 isolates were clustered into the *Rhodobacteraceae* family of α-proteobacteria and the *Pseudomonadaceae* family of γ-proteobacteria. To our knowledge, this is the first report detailing the isolation of organisms of *Pseudomonas stuzeri* sp. and the *Rhodobacteraceae* family with 2-haloacid dehalogenase activity from marine sponges.

Keywords Bacteria · Marine sponge · 2-haloacid dehalogenase · Salt tolerance · Enantioselectivity

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Introduction

The 2-haloacid dehalogenases catalyze the dehalogenation of 2-haloalkanoic acids to produce 2-hydroxyalkanoic acids. Three groups of 2-haloacid dehalogenases have been described based on their substrate and stereochemical specificities (Fetzner and Lingens 1994). R-2-haloacid dehalogenases and S-2-haloacid dehalogenases specifically act on R and S enantiomers of 2-haloacid, respectively. R,S-2-haloacid dehalogenases are able to dehalogenate both enantiomers of 2-haloacids. 2-haloacid dehalogenase is important to environmental and industrial technologies (Kurihara and Esaki 2008; Swanson 1999). Microorganisms capable of producing 2-haloacid dehalogenase have frequently been isolated from soil, but very rarely from marine environments or organisms (Olaniran et al. 2004; Tsang and Sam 1999).

The ocean is a vast reservoir of organohalogen compounds and microorganisms that have the ability to degrade such compounds (Gordon 1998; Smidt and de Vos 2004). Marine sponges (phylum *Porifera*) are sessile filter feeders that acquire a wide range of particulate matter, including organic matter, bacteria and phytoplankton (Larsen and Riisgard 1994; Riisgard and Larsen 1995). The ability of sponges to concentrate large numbers of microorganisms

make them excellent candidates for isolating novel marine bacteria that may be used for research involving drug discovery or novel industrial enzymes (Taylor et al. 2007).

Many aquatic animal species are resistant to toxicity and have the ability to generate an economic return following remediation activities (Gifford et al. 2007). Indeed, the sponges have been reported to be suitable bioremediators for organic matter and microbial contaminants (Donovan et al. 2009; Fu et al. 2006; Fu et al. 2007; Longo et al. 2010; Zhang et al. 2010). However, investigations on removing organohalogen compounds are rare. As an earlier study reported, degradation of halogenated chemicals occurred within the marine sponge *Aplysina aerophoba* (Ahn et al. 2003). Antibiotic inhibition of dehalogenation activity indicated that the microbes, not the sponge, were responsible for the dehalogenation activity; furthermore, a large population of anaerobic dehalogenating bacteria was found inside the sponge body. Therefore, it is rational to investigate the dehalogenase-producing bacteria isolated from marine sponges in an attempt to identify potential new bacterial strains for bioremediation and other purposes.

The sponge *H. perlevis* inhabits the estuarine intertidal area of the Chinese Yellow Sea. Based on earlier reports, high microbial diversity, especially of Actinobacteria, was found within this sponge (Dharmaraj 2010; Sun et al. 2010; Xin et al. 2008; Xin et al. 2009; Zhang et al. 2006; Zhang et al. 2008; Zheng et al. 2005). Our preliminary work showed that the sponge was a good source for the dehalogenase producing bacteria isolation (Huang et al. 2010). Here we report the isolation of 2-haloacid degrading bacteria from *H. perlevis*. The isolates were initially identified by 16S rRNA sequence comparison, and they were then tested for potential dehalogenation activity. Such activity may be useful in furthering industrial and environmental applications.

Materials and methods

Sponge collection

The sponge *H. perlevis* was collected manually from the intertidal coast of the Chinese Yellow Sea near Dalian City. This area in particular is highly polluted with a wide range of chlorinated intermediates and products discharged from surrounding industrial sites. A tissue suspension was prepared by grinding approximately 1 g of sponge tissue in 10 ml of sterile, natural seawater with a mortar and pestle.

Medium and culture condition

The enrichment medium was minimal medium (Brunner et al. 1980) supplemented with 0.04 g bromothymol blue/l (as pH indicator) and 20 ml of 1 M 2-CPA solution. The indicator agar was prepared as enrichment medium supplemented with 1.5% (w/v) agar. The 2-CPA medium (pH 7.0) consisted of enrichment medium supplemented with 1.5 g KH_2PO_4/l and 10.5 g $Na_2HPO_4 12H_2O$/l. The 2-CPA solution was prepared at 1 M concentration, neutralized to pH 7.0 using 1 M NaOH and sterilized using a Millipore filter (Type HA, pore size 0.22 μm). All cultures were incubated in the dark at 28°C , and all cultures in liquid medium were carried out in an orbital shaker at 200 rpm.

Isolation and screening

Enrichment cultures were made by inoculating 1 ml of tissue suspension into 250 ml flasks containing 100 ml of enrichment medium. The cultures were grown at 28°C with 200 rpm shaking. The degradation of 2-CPA in the medium was monitored using the pH indicator method. After being sub-cultured for 3 times, serial dilutions were plated onto indicator plates. The isolates showing significant growth and yellow color were inoculated into liquid 2-CPA medium. After 3 days, the degradation of 2-CPA in the culture was measured using HPLC. The isolates that degraded more than 50% of the total 2-CPA were purified and selected for further research.

Degradation under high salinity

To test each isolate's ability to degrade 2-CPA under high salinity, the isolates were inoculated on 2-CPA agar, which was prepared as 2-CPA medium supplemented with 1.5% (w/v) agar and the following salt concentrations: 0, 3, 8 and 15%. The growth of each isolate on the medium was observed.

Substrate selectivity

The substrate selectivity of each isolate was assayed by investigating the growth of the isolate on different mineral-based mediums using 1,2-dichloroethane, 3-chloro-1, 2-propanediol, 2,2-dichloropropionic acid and 2,4,6-trichlorophenol as sole carbon sources. The four kinds of compounds were supplemented (0.5 g carbon per liter) in the mineral-based medium.

Investigation of stereoselectivity and activity

The cells from each isolate were cultured at 28°C in 2-CPA liquid medium containing 20 mM 2-CPA. Cells were harvested by centrifugation and suspended in 100 mM phosphate buffer (pH 7.5) containing EDTA (1 mM) and dithiothreitol (0.01% w/v). The re-suspended cells were disrupted by ultrasonication (400 W for 20 min), and a

crude enzyme extract was obtained by centrifugation (15 min at 12,000 g and 4°C).

The stereoselectivity of the isolates was estimated by separately measuring the initial rate of dehalogenation of two pure enantiomers under the same conditions. The assay mixture (1 ml) contained 1 µl 2-CPA, 50 mM Tris-H_2SO_4 buffer, 0.01% w/v DTT and 100 µl of crude enzyme extract. After incubation at 30°C for 20 min, the reaction was terminated by the addition of 10 µl of phosphoric acid (1% v/v).

The dehalogenase activities of the isolates were determined using a 1 ml assay mixture containing 10 mM substrate, 100 mM phosphate buffer (pH 7.3), 0.01% w/v dithiothreitol (DTT) and 200 µl of crude enzyme extract. After incubation at 30°C for 20 min, the reaction was terminated by the addition of 10 µl of phosphoric acid. One unit of the enzyme was defined as the amount of enzyme that catalyzes the dehalogenation of 1 µmol of substrate/min. The protein assay was done using the Bradford method (Bradford 1976).

Analysis method

The 2-haloacid dehalogenase activity of each isolate was determined by measuring the consumption of substrate using a reverse phase HPLC method (Rampazzo 1986). The conditions for the analysis were as follows: column, Hypersil GOLD column (8 µm, 250 mm × 21.2 mm i.d.), (Thermo, America); mobile phase, acetonitrile–water (20:80) (pH 2.1) containing 10.5 ml phosphoric acid (1%, w/v); flow-rate, 1.0 ml/min; wavelength, 210 nm; and temperature, 25°C .

Phylogenetic diversities of the isolates

To sequence the 16S rRNA gene of each isolate, total genomic DNA was extracted from bacteria using a simple method (Mahuku 2004). The 16S rRNA gene was amplified with two universal primers, 8F (5′-AGAGTTTGATC CTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTAC GATCC-3′). PCR products were purified and sequenced by TaKaRa Biotechnology Co., Ltd. (Dalian, China). Related sequences were obtained from the GenBank database using the BLAST search program, and sequences were aligned with CLUSTAL W software (Thompson et al. 1994). Evolutionary distance matrices were calculated using the algorithm of Jukes and Cantor with the DNADIST program within the PHYLIP package (Jukes and Cantor 1969). A phylogenetic tree was constructed using the neighbor-joining method (Felsenstein 1985) as implemented within the NEIGHBOR program of the same package (Saitou and Nei 1987).

Results

Isolation and screening

To further utilize the enrichment media in this study, a pH indicator medium was developed. In this medium, 2-CPA degradation yields a linear pH change that results in a linear color change, which can be measured at an optical density of 616 nm (OD616). As shown in Fig. 1, the OD616 value of the enrichment medium decreased from 1.08 to 0.408 when the 2-CPA in the enrichment media was degraded by approximately half of its original total (10 mM). Thus, when the OD616 value of the enrichment medium decreased to approximately 0.4, the enrichment medium was sub-cultured.

Using this method, a total of 139 isolates that were able to use 2-CPA as the sole carbon source were isolated. Among them, 28 isolates that consistently showed deep yellow color and different morphological characteristics on the indicator agar were selected for further screening in liquid 2-CPA medium. To investigate the 2-CPA degradation abilities of the isolates in liquid 2-CPA medium, an HPLC method was developed and adapted to analyze the consumption of 2-CPA in the medium. After being cultured in 2-CPA medium for 3 days, the residual 2-CPA in the medium was analyzed. As shown in Table 1, the degradation rates of the isolates ranged from 36.3 to 100%. Among the 28 isolates, 11 isolates showed a degradation rate greater than 50%. Among the 11 isolates, the DEH84 and DEH130 isolates degraded the 2-CPA completely. The 11 isolates with high 2-CPA degradation rates (above 50%)

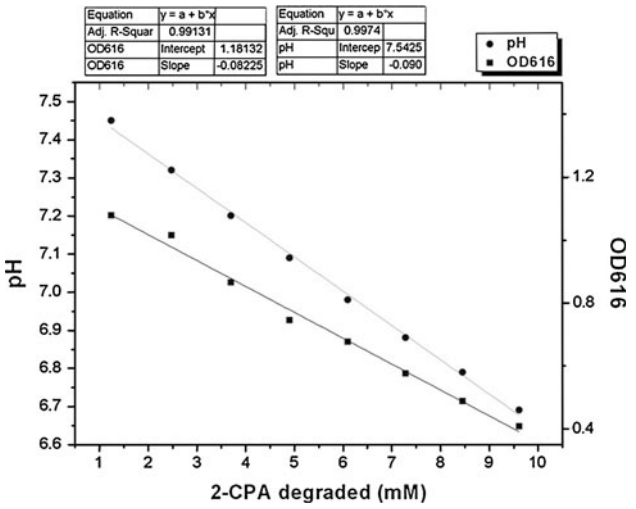

Fig. 1 The pH and OD616 value showing 2-CPA degradation profile in the enrichment medium. The degradation of 2-CPA was simulated by the presence of hydrochloride and lactic acid at different concentrations, corresponding to products released by 2-CPA degradation

Table 1 The 2-CPA degradation rate of the strains in 2-CPA medium supplemented with 20 mM 2-CPA after 3 days of incubation at 28°C

No. of strains	Degradation rate (%)	Strain number	Degradation rate (%)	Strain number	Degradation rate (%)
DEH 129	36.3	DEH 6	41.7	DEH 99	58.9
DEH 31	36.4	DEH 10	44.9	DEH 90	70.5
DEH 18	38	DEH 27	44.1	DEH 78	72.6
DEH 62	38.1	DEH 14	44.3	DEH 115	75.7
DEH 69	38.3	DEH 19	44.4	DEH 72	84.7
DEH 89	39	DEH 32	45.4	DEH 125	89.5
DEH 123	39.2	DEH 8	48	DEH 84	100
DEH 9	40.5	DEH 138	54	DEH 130	100
DEH 56	41	DEH 116	56.9		
DEH 13	41.4	DEH 66	58.6		

were selected and stored as glycerol stocks at −70°C after purification on 2216E marine agar.

Degradation under high salinity

With regard to degradation abilities of the 11 isolates under high salinity, the growth of each of these isolates on 2-CPA agar with different salinity was investigated. As shown in Table 2, all 11 of the isolates were able to grow on media containing 0–3% salt, but isolate DEH116 could not grow on 0% salt medium. Isolates DEH66, DEH84, DEH90, DEH99, DEH125, DEH130 and DEH138 were able to grow on 8% salt. Among these seven isolates, four isolates (DEH 66, DEH 99, DEH125 and DEH138) grew on 15% salt. No isolate was able to grow on 20% salt.

Substrate specificity

The substrate specificity of the isolates was investigated using four kinds of common organohalogen compounds of different structures. As shown in Table 3, eight of the 11 isolates (DEH66, DEH84, DEH90, DEH99, DEH115, DEH116, DEH130 and DEH138) utilized all four kinds of organohalogen compounds. The other three isolates utilized only two or three compounds. The DEH78 isolate could not utilize 2,2-dichloropropionic acid. The DEH72 isolate could not utilize 2,2-dichloropropionic acid and 2,4,6-trichlorophenol. The DEH125 isolate could not utilize 2,2-dichloropropionic acid and 3-chloro-1,2-propanediol.

Table 2 Growth of the strains on 2-CPA medium supplemented with different concentration of NaCl after 14 days of incubation at 28°C

No. of strains	Salinity of the medium (weight to volume ratio)			
	0	3%	8%	15%
DEH 66	+	+	+	+
DEH 72	+	+	−	−
DEH 78	+	+	−	−
DEH 84	+	+	+	−
DEH 90	+	+	+	−
DEH 99	+	+	+	+
DEH 115	+	+	−	−
DEH 116	−	+	−	−
DEH 125	+	+	+	+
DEH 130	+	+	+	−
DEH 138	+	+	+	+

+, normal growth; −, no growth

Investigation of enantioselectivity

The enantioselectivity of the isolates was investigated by analysis of the isolates' dehalogenase activities against different isomers of 2-CPA and expressed using the value of $[(VR - VS)/(VR + VS)] \times 100\%$. The positive value represents R-selectivity, the negative value represents S-selectivity and the absolute value represents the strength of the selectivity. As shown in Table 4, the DEH116, DEH130 and DEH138 isolates showed R-selectivity, whereas the other isolates showed S-selectivity. The DEH99 and DEH138 isolates exhibited the best selectivity towards S-CPA and R-CPA, respectively, which could only degraded one isomer of 2-CPA in this study.

Dehalogenase assay in crude extract

Among the 11 isolates, the DEH99 and DEH138 isolates exhibited the best enantioselectivity towards S-CPA and R-CPA, respectively, as well as the DEH84 and DEH130 isolates could totally degrade 2-CPA during the screening phase. The dehalogenase activities of these isolates were analyzed. The dehalogenase activity of DEH84 against racemic CPA, DEH99 against S-CPA, DEH138 against R-CPA, and DEH130 against racemic CPA were 0.16 U/mg, 0.06 U/mg, 0.12 U/mg, and 0.19 U/mg, respectively.

Table 3 Growth of the strains on mineral-based medium supplemented with four kinds of compounds (0.5 g of carbon per liter) after 7 days incubation at 28°C

No. of strains	1,2-dichloroethane medium	3-chloro-1,2-propanediol medium	2,2-dichloropropionic acid medium	2,4,6-Trichlorophenol medium
DEH 66	+	+	+	+
DEH 84	+	+	+	+
DEH 90	+	+	+	+
DEH 99	+	+	+	+
DEH 115	+	+	+	+
DEH 116	+	+	+	+
DEH 130	+	+	+	+
DEH 138	+	+	+	+
DEH 78	+	+	−	+
DEH 72	+	+	−	−
DEH 125	+	−	−	+

+, normal growth; −, no growth

Table 4 Enantioselectivity estimation of the isolates

Strain no.	[(VR − VS)/(VR + VS)] × 100% (%)
DEH 138	100
DEH 116	63.4
DEH 130	38.1
DEH 78	−27.4
DEH 84	−42.9
DEH 72	−44.9
DEH 125	−51.5
DEH 66	−60
DEH 115	−72.4
DEH 90	−85.2
DEH 99	−100

The assay mixture (1 ml) contained 1 μl 2-CPA, 50 mM Tris–H_2SO_4 buffer, 0.01% w/v DTT and 100 μl of crude enzyme extract. Incubation at 30°C for 20 min

VS: initial velocity of degradation against S-CPA; *VR*: initial velocity of degradation against R-CPA

Phylogenetic diversities of the isolates

Phylogenetic diversities of the isolates were performed by16S rDNA sequence analysis. Sequences with the highest similarity to the 11 isolates were downloaded from the RDP database, and the phylogenetic trees were constructed (Figs. 2, 3). Based on comparison of the 16S rRNA genes of the isolates with previously published 16S rRNA gene sequences, the 11 isolates were clustered into two groups. Isolates of group I belong to the *Rhodobacteraceae* family of a-proteobacteria, whereas isolates of group II belong to the *Pseudomonadaceae* family of g-proteobacteria.

Group I is composed of six sequences (as shown in Fig. 2) that are further divided into two subgroups. In

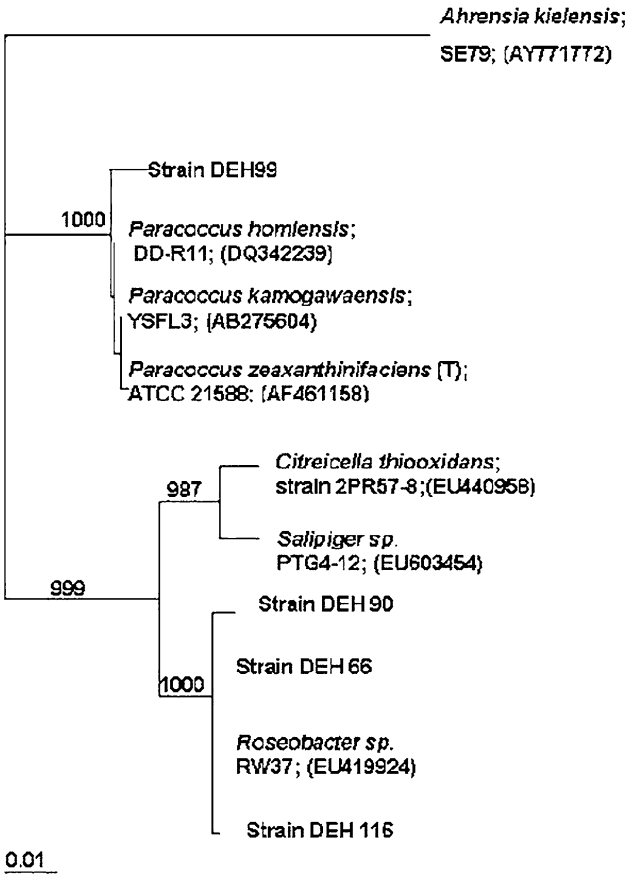

Fig. 2 Neighbor-joining tree of strains DEH66, DEH90, DEH99 and DEH116 based on 16S rRNA gene sequences. The sequence of *Ahrensia kielensis*; SE79; (AY771772) was used as the out group. Scale Bar 0.01 indicates sequence divergence

subgroup I, the DEH66 and DEH90 isolates share 99.5 and 99.2% sequence similarity with *Roseobacter* sp. RW37 EU419924, respectively. The DEH116 isolate shares

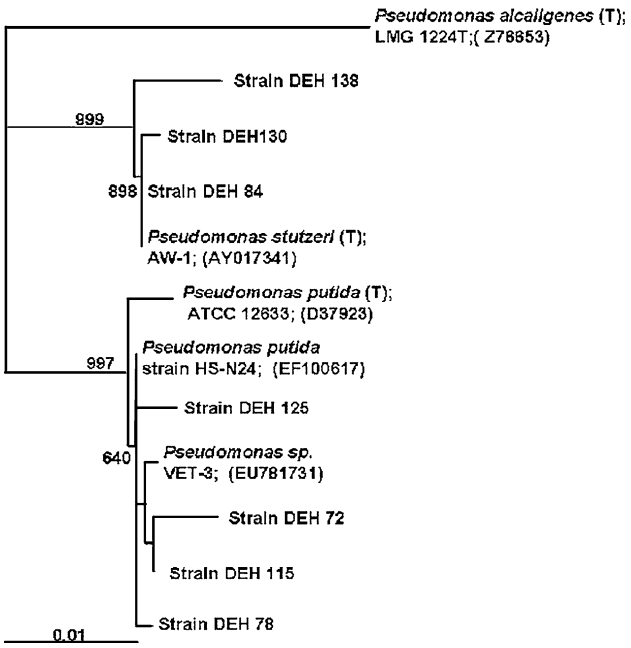

Fig. 3 Neighbor-joining tree of strains DEH72, DEH78, DEH84, DEH115, DEH125, DEH130 and DEH138 based on 16S rRNA gene sequences. The sequence of *Pseudomonas alcaligenes* (T) LMG 1224T;(Z76653) was used as the out group. Scale Bar 0.01 indicates sequence divergence

98.8% sequence similarity with *Roseobacter* sp. RW37 EU419924. These three isolates were identified as *Roseobacter* sp. The DEH99 isolate shares 99.1% sequence similarity with *Paracoccus homiensis* DD-R11 and was identified as *P. homiensis* sp.

Group II is composed of 14 sequences (as shown in Fig. 3) that are further divided into two subgroups. In subgroup I, the DEH130 and DEH84 isolates share 98.1 and 98.5% sequence similarity to *Pseudomonas stuzeri* sp, respectively. The DEH138 isolate shares 97.5% sequence similarity with *Pseudomonas stuzeri* sp. The three isolates were identified as *Pseudomonas stuzeri* sp. In subgroup II, the DEH72 and DEH115 isolates shared 99.7% sequence similarity with *Pseudomonas putida* sp, and the DEH78 and DEH125 isolates shared 98.1 and 98.2% sequence similarity to *Pseudomonas putida* sp, respectively. These four isolates were identified as *Pseudomonas putida* sp.

Nucleotide sequence accession numbers

The 16S rRNA gene sequences of the isolates were deposited in the GenBank nucleotide sequence databases using the following accession numbers: DEH66 (FJ713773);DEH72 (FJ713774); DEH78 (FJ713775); DEH84 (FJ713783); DEH90 (FJ713776); DEH99 (FJ713782); DEH115 (FJ713777); DEH116 (FJ713778); DEH125 (FJ713779); DEH130 (FJ713780) and DEH138 (FJ713781). The isolates with potential use had been submitted to China Center of Industries Culture Collection using the following reference numbers: DEH84 (CICC 10428); DEH99 (CICC 10429); DEH130 (CICC 10430); DEH138 (CICC 10431).

Discussion

In comparison with isolating 2-haloacid degrading organisms from soil, one challenge for isolating 2-haloacid degrading organisms from marine sponges is to detect the increasing chlorine (Cl^-) in seawater, which has a high chlorine Cl^- concentration (Bergmann and Sanik 1957). In this study, the increasing concentration of H^+ or decreasing concentration of 2-CPA due to 2-CPA degradation were analyzed, but the Cl^- released was not analyzed as in other studies (NardiDei et al. 1997; Van der Ploeg et al. 1991).

In this study, the pH indicator method was used to facilitate the preparation of enriched media. Although the accuracy of the pH indicator method is compromised because the pH of the medium is not only affected by degradation of 2-CPA, this test was appropriate for this study. To investigate the ability of each isolate to degrade 2-CPA in liquid medium, an HPLC method was adapted to analyze the consumption of 2-CPA in the medium. The HPLC method was accurate and suitable for high throughput screening.

The 2-haloacid dehalogenases are involved in the degradation pathways of α-dichloropropionate (Marchesi and Weightman 2003), 1,2-dichloroethane (Janssen et al. 1994) and γ-hexachlorocyclohexane KEGG. These organohalogen compounds are commonly found in contaminated environments. The isolation of marine sponge microorganisms that are able to degrade organohalogen compounds may be useful in designing new bioremediation systems because these isolates are adapted to the marine environment. As shown by the results reported here, most of the isolates demonstrated high salt tolerance and wide degradation spectra. The isolates DEH130 and DEH84 can degrade 2-CPA completely and utilize all four kinds of organohalogen compounds. In addition, they can degrade 2-CPA and grow under salt concentrations ranging from 0 to 8% (weight to volume ratio). The dehalogenase activity of DEH84 and DEH130 were 0.16 U/mg and 0.19 U/mg, respectively, which is equivalent to the activity of 2-haloacid degrading bacteria in soil (Liu et al. 1994; Motosugi et al. 1982). The isolation of these efficient organohalogen compounds degrading bacteria suggest that abundant sponge populations could have important roles on removing organohalogen pollution from the habitats they reside in. And these features make the isolates good candidates for bioremediation in marine environments and the treatment of hypersaline wastewater.

In addition to applications in waste treatment and bioremediation, the stereoselectivity of 2-haloacid dehalogenases can be used to selectively dehalogenate one of the isomers of 2-CPA from its racemic mixture, producing chiral 2-CPA and lactic acid, which are chiral feedstock chemicals for the production of herbicides and pharmaceutical products (Fetzner and Lingens 1994; Kurihara and Esaki 2008). In this study, all isolates showed enantioselectivity to some extent. The DEH99 and DEH138 isolates are S-specific and R-specific, respectively. The dehalogenase activity of DEH99 against S-CPA is 0.06 U/mg, whereas the dehalogenase activity of DEH138 against R-CPA is 0.12 U/mg. In addition, the high salt tolerances of these isolates allow them to degrade 2-CPA under high 2-CPA sodium concentrations. In return, the high concentration of 2-CPA improves the reaction and production recovery efficiency. Identification of enzymes with improved industrial properties is the main objective of dehalogenase research. The enantioselectivity specificity and salt tolerance of the DEH99 and DEH138 isolates make them promising candidates for development in industrial processes.

Organisms of the genera *Xanthobacter*, *Burkholderia* and *Pseudomonas* that possess 2-CPA degradation ability have been isolated from terrestrial soil (Jones et al. 1992; Tsang and Sam 1999; Van der Ploeg et al. 1991), but research in a marine environment or studies on the isolation of potential isolates from marine sponges has not been conducted so far. To our knowledge, this is the first report on the isolation of 2-CPA degrading organisms from a marine environment. The results of this study revealed a difference in biodiversity of marine 2-CPA degrading organisms and terrestrial organisms. In this study, we report for the first time that organisms of *Pseudomonas stuzeri* sp and the *Rhodobacteraceae* family are 2-CPA degrading bacteria. In addition, the isolates from the *Rhodobacteraceae* family showed a wider substrate range and higher salt tolerance than the isolates of *Pseudomonas putida* sp. The latter are renowned for their ability to metabolize an extensive number of substrates, such as chlorinated hydrocarbons (Field and Sierra-Alvarez 2008; Sahasrabudhe and Modi 1987). The *Rhodobacteraceae* family, which belongs to alphaproteobacteria, comprises one of the largest fractions of heterotrophic marine bacteria (Cottrell and Kirchman 2000; Hagstrom et al. 2002). The results of this study imply that alpha-proteobacteria might play a significant role in the breakdown of recalcitrant organic pollutants in marine environments.

In conclusion, the isolation of 2-CPA degrading bacteria from the sponge *Hymeniacidon perlevis* resulted in the isolation of a total of 139 2-haloacid degrading bacteria that were screened using novel approaches. Eleven isolates with high 2-CPA degrading ability were characterized. Most of these isolates exhibited extensive substrate utilization abilities, high salt tolerance and good enantioselectivities. Hence, these isolates might be very useful in bioremediation and industrial applications. Finally, this study is the first to identify organisms of *Pseudomonas stuzeri* sp or the *Rhodobacteraceae* family as 2-CPA degrading bacteria. These significant findings will contribute to a better understanding of the distribution and evolution of 2-CPA degrading bacteria and suggest that the marine sponge *Hymeniacidon perlevis* may be a useful source for the isolation of 2-CPA degrading bacteria.

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