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ORIGINAL PAPER

Isolation, gene detection and solvent tolerance of benzene, toluene and xylene degrading bacteria from nearshore surface water and Pacific Ocean sediment

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Abstract BTX (benzene, toluene and xylene) degrading bacteria were isolated from Pacific Ocean sediment and nearshore surface water. In the seawater near a ferry dock, degrading bacteria of a relatively wide diversity were detected, including species of *Pseudomonas*, *Rhodococcus*, *Exiguobacterium* and *Bacillus*; while species of *Bacillus* only have been detected from the deep-sea sediment. Most of the isolates showed degradation to more than one compound. Generally better growth was obtained with *p*-xylene and ethylbenzene than with the other two. All the bacteria could tolerate and grow with the compounds at 5–20% (v/v). Both benzene and toluene degradation related genes had been successfully PCR cloned from the isolates of nearshore water, the detected benzene dioxygenase gene was identical among all the species and close to its soil counterpart. However, they were not detected in all the isolates from deep sea. Results in this report suggested that BTX degrading bacteria widely spread in marine environments and they might be of potentials in biotreatment of BTEX in saline environments.

Keywords Benzene · Toluene · Xylene · Biodegradation · Solvent tolerance · Degrading gene · Marine bacteria

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Introduction

BTEX is the acronym for a group of monocyclic aromatic hydrocarbons that includes benzene, toluene, ethylbenzene and xylene. BTEX is a known carcinogen and poses a risk to human health and other kinds of life (Edwards and Grbic-Galic 1992; Rael et al. 1995; Ball and Reinhard 1996; Fang et al. 2004). In addition, it can nonspecifically break cell membrane integrity, increase permeability and lead to lysis (Matsumoto et al. 2002). Thus, Benzene and toluene have been placed in the list of priority pollutants (Clean Water Act, section 307. More details can be found at: http://www.scorecard.org/chemical-groups/one-list.tcl?short_list_name=pp).

BTEX widely exists in petroleum and related products. Despite its volatility, it constitutes the major components of petroleum in the water-soluble fraction (Saeed and AL Mutairi 1999). In contrast with the tremendous input of petroleum into marine environments annually, the fate and effect of BTEX in marine environment are far to be known.

Many BTEX degraders have been isolated in different environments, such as soil degraders like species *Pseudomonas* (Arenghi et al. 2001), *Rhodococcus* (Kim et al. 2002; Taki et al. 2007), *Marinobacter* (from a brine soil, Nicholson and Fathepure 2004) and *Acinetobacter* (Wang and Shao 2006). In addition, *Alcaligenes*, *Brevibacterium*, *Nocardia*, *Bacillus*, *Bordetella*, *Arthrobacter*, *Bradyrhizobium*, *Acidovorax*, *Agrobacterium*, *Aquaspirillum*, *Variovorax* and *Stenotrophomonas* were also detected as BTEX degraders in soil (Hendrickx et al. 2006a, b). From sewage and fresh water, the BTEX degraders were reported as *Ralstonia* (Kahng et al. 2000), *Microbacterium* (Cavalca et al. 2004), *Mycrobacterium* (Cavalca et al. 2004), *Azoarcus* (Cavalca et al. 2004; Mohamed et al. 2001), *Thauera* (Yoshifumi et al. 2004), *Burkholderia* (Johnson and Olsen 1997) and

Sphingomonas (Fredrickson et al. 1995). Moreover, BTEX degradation in underground water and under anaerobic conditions is also actively investigated (Chakraborty and Coates 2004; Hendrickx et al. 2005; Jahn et al. 2005; Botton and Parsons 2006). However, few reports have been focused on marine BTEX degradation and no bacteria have been confirmed as potential degraders of BTEX, except *Cycloclasticus* (Wang et al. 1996) and *Alcanivorax* (Dutta and Harayama 2001).

In this report, BTX (benzene, toluene and xylene) degrading bacteria in nearshore surface water and deep sea sediment were enriched and isolated, with an aim to learn the biodiversity of BTX degrading micro-organisms therein.

Materials and methods

Samples, chemicals and media

West Pacific Ocean sediments were sampled in February 2002 with a multicore sampler; nearshore surface water was taken from Xiamen Ferry Dock (on the side of a busy international waterway, west bank of Taiwan Straits) in May 2004. Surface water and sediment samples were stored at 4°C prior to use. Benzene, toluene, ethylbenzene and *p*-xylene all are in purity of 99.5%, filtration-sterilized and used as the sole carbon and energy source to enrich degrading bacteria.

The liquid mineral medium (MM) contained NaCl 30 g l^{-1}, $MgSO_4 \cdot 7H_2O$ 7.0 g l^{-1}, NH_4NO_3 1.0 g l^{-1}, KCl 0.7 g l^{-1}, KH_2PO_4 2.0 g l^{-1}, Na_2HPO_4 3.0 g l^{-1} and the trace elements $CaCl_2$ 0.02 mg l^{-1}, $FeCl_3 \cdot 6H_2O$ 0.5 mg l^{-1}, $CuSO_4$ 0.005 mg l^{-1}, $MnCl_2 \cdot 4H_2O$ 0.005 mg l^{-1}, $ZnSO_4 \cdot 7H_2O$ 0.1 mg l^{-1} and pH 7.4.

HLB medium was modified from Luria–Bertani (LB) medium, with 30 g l^{-1} NaCl.

Selective plates were made by adding 1.5% (wt/ml) agar in MM medium. Benzene, toluene or xylene was provided in the form of vapor by slow spreading from a strip of filter paper from a capped 200 µl Eppendorf tube in Petri dishes.

Enrichment and isolation of benzene, toluene or xylene-degrading bacteria

About 5 ml surface seawater or 1 g west Pacific Ocean sediment sample was added to 50 ml of sterile MM medium in a 250 ml silicone plug-capped flask. Benzene, toluene or xylene was added in sterile MM medium by 0.1% (v/v). The flasks of surface water and sediment treatments were incubated at 28 and 18°C, respectively, with constant shaking at 150 rpm. After 1 week of enrichment, 1 ml culture was transferred into a fresh medium and further incubated. Growth was measured by optical density at 600 nm (OD_{600}) with a spectrophotometer (UltroSpec UV-2100, Amersham Pharmacia). After four subcultures, an appropriate dilution of the culture was spread onto the selective plates. Benzene, toluene or xylene degrading bacteria were screened out by the appearance of colonies.

Phylogenetic characterization

Nearly full-length fragments of 16S rRNA gene were amplified with the following primer pair: forward (27R-ACGGCTACCTTGTTACGACT) and backward (1502F-AGAGTTT GATCCTGGCTCAG). Polymerase chain reaction products were separated by agarose gel electrophoresis and purified using a DNA purification kit (UNIQ-10, made by Shanghai Sangon), and then cloned into pMD18-T vector and transformed into competent *Escherichia coli* DH-5α. The inserted fragments were sequenced with a model 377, automated DNA sequencer using a Big-Dye Terminators Cycle Sequencing kit (Applied Biosystems).

The 16S rRNA gene sequences were blasted with sequences in the GenBank database. Phylogenetic trees were constructed with the software DNAMAN (version 5.1, Lynnon Biosoft).

Growth requirements on temperature, salinity and pH

Bacteria isolated from nearshore surface water were grown at 28°C and those from deep-sea sediments were grown at 18°C, 150 rpm, in MM medium containing 0.1% (v/v) of benzene, toluene or xylene. In late exponential phase, cells were harvested by centrifugation, washed twice in sterile MM and resuspended in one-tenth volume of medium. This cell suspension was used as inoculum for subsequent experiments. Bacteria were cultivated at a series of temperatures including 4, 18, 28 and 37°C in MM medium with benzene, toluene or xylene as the sole carbon source and the growth rate was calculated by measuring OD_{600} of the culture. For optimal salinity determination, NaCl concentration was set from 0 to 6% (wt/ml); optimal pH was tested from pH 4 to 10 with the medium of 3% salinity.

Biodegradation to various aromatic compounds

Inoculum of deep-sea strains prepared as above was inoculated into 100 ml of MM medium containing each of the

aromatic compounds (1%, v/v) in a 250 ml bottle with silicone rubber stoppers. The used aromatic compounds were benzene, toluene, xylene and ethylbenzene. Cells were cultivated with shaking (150 rpm) at 18°C. The growth was monitored by measuring OD_{600}.

In the test with bacteria from dock water, it was conducted by inoculating a single clone from plate into MM medium with 20% (v/v) each compound of BTEX and cultivated for 3 days at 28°C, 180 rpm. Each treatment above was in triplication.

Solvent-tolerance examination

Pure isolates were incubated in HLB media spiked with 0, 5, 10, 15, 20, 30, 50, 70 and 90% (v/v) toluene or xylene solvent, respectively. Cell growth was monitored by measuring OD_{600} of 5 days culture. Non-inoculated treatments were used as controls.

Oxygenase gene detection by PCR from all the isolates

Bacterial genomic DNA was used as a template for PCR to clone the BTX oxygenase genes from the obtained isolates. The primers of benzene dioxygenase gene were Ben-F (5′-TCGTCGTCAGACACTACGTA-3′) and Ben-R (5′-AATCTGATGCTTGCCATCATGG-3′), designed based on the alpha subunit gene *bedC1* of benzene dioxygenase. Primers for toluene monoxygenase gene (TMOA-F and TMOA-R, TOL-F and TOL-R, TBMD-F and TBMD-R), primers for xylene monoxygenase gene (TouA-F and TouA-R, XYLA-F and XYLA-R), and primers for detecting genes involved in a direct dioxygenase attack of benzene, toluene and ethylbenzene (TODC1-F and TODC1-R), were synthesized according to those described by Hendrickx et al. (2006a, b). The sequence of these primers is listed below. More details about the primers were described by Hendrickx et al. (2006b).

TMOA-F: 5′-CGAAACCGGCTT(C/T)ACCAA(C/T)ATG-3′
TMOA-R: 5′-ACCGGGATATTT(C/T)TCTTC(C/G)AGCCA-3′
TOL-F: 5′-TGAGGCTGAAACTTTACGTAGA-3′
TOL-R: 5′-CTCACCTGGAGTTGCGTAC-3′
TBMD-F: 5′-GCCTGACCATGGATGC(C/G)TACTGG-3′
TBMD-R: 5′-CGCCAGAACCACTTGTC(A/G)(A/G)TCCA-3′
TouA-F: 5′-AAGACCTATCCSGARTACGT-3′
TouA-R: 5′-GGCTGGATCWGRCCTGCSAGGAA-3′
X YLA-F: 5′-CCAGGTGGAATTTTCAGTGGTTGG-3′
XYLA-R: 5′-AATTAACTCGAAGCGCCCACCCCA-3′
TODC1-F: 5′-CAGTGCCGCCA(C/T)CGTGG(C/T)ATG-3′
TODC1-R: 5′-GCCACTTCCATG(C/T)CC(A/G)CCCCA-3′

The PCR program of *bedC1* gene was set as: 94°C for 4 min and 30 cycles at 94°C for 1 min, 48°C for 1 min and 72°C for 1 min, and with an extension at 72°C for 10 min. The PCR programs of other genes were according to those described by Hendrickx et al. (2006b). The products were purified, cloned and sequenced as described above. Alignment analysis was constructed with DNAMAN software.

Nucleotide sequence accession numbers

The accession numbers of the isolates 16S rRNA gene from deep sea on GenBank are from DQ451096 to DQ451100; those from nearshore water are from EF683118 to EF683123. The toluene dioxygenase gene sequence from isolate LE9 and LUN2 were deposited as EF683124 and EF683125, respectively; those from isolate LB1, LJ2 LJ8, LE2, LE9 and LUN2 were assigned with accession No. as from EF683126 to EF683131.

Results

Isolation of BTX degrading bacteria from Xiamen Ferry Dock and Pacific Ocean sediment

Six bacteria were obtained from surface seawater of Xiamen Ferry Dock and five from the Pacific Ocean sediment as BTX degraders, separately. Among the six bacteria from the surface water: the isolate LB1 was isolated from enrichment with benzene as the sole carbon source, LJ2 and LJ8 from toluene, and LE2, LE9 and LUN2 from xylene (Table 1). Among the five isolates from deep sea, JB5 and JB7 were isolated from enrichment with toluene as the sole carbon source, EJB1, EJB5 and EJB8 were isolated from xylene enrichment (Table 1). No benzene degraders were obtained from deep sea.

Characterization of the obtained BTX degrading isolates

16S rDNA sequence analyses showed that the bacteria from dock water belonged to four genera, *Pseudomonas*, *Rhodococcus*, *Exiguobacterium* and *Bacillus* (Fig. 1a). Among them, LUN2 and LE9 belonged to *Pseudomonas*, which degraded xylene. Isolate LUN2 was close to *Pseudomonas putida* (AF291048) (99%), LE9 was close to

Table 1 BTX degrading bacteria isolated from nearshore water and deep-sea sediment

Isolates (MCCC No.)	Solvent[a]	Closest strain from NCBI	Identity by 16S rDNA (%)	Accession No. in GenBank
LB1 (1A00328)	Benzene	*Bacillus subtilis* strain MO5 (AY553098)	99	EF683118
LE2 (1A00331)	Xylene	*Rhodococcus rhodochrous* strain DSM43274T (X80624)	99	EF683121
LE9 (1A00290)	Xylene	*Pseudomonas stuzeri* 11C2 (AJ270452)	99	EF683122
LJ2 (1A03188)	Toluene	*Rhodococcus pyridinivorans* strain R04 (AF459741)	99	EF683119
LJ8 (1A00330)	Toluene	*Exiguobacterium gaetbuli* strain TF-16(AY594264)	99	EF683120
LUN2 (1A00332)	Xylene	*Pseudomonas putida* (AF291048)	99	EF683123
JB5 (1A03189)	Toluene	*Bacillus mojavensis* (AB021191)	99	DQ451099
JB7 (1A00327)	Toluene	*Bacillus mojavensis* (AB021191)	99	DQ451100
EJB1 (1A03190)	Xylene	*Bacillus fusiformis* SW-89 (AY907676)	99	DQ451096
EJB5 (1A00324)	Xylene	*Bacillus fusiformis* SW-89 (AY907676)	99	DQ451097
EJB8 (1A03187)	Xylene	*Bacillus cereus* LRN (AY138275)	99	DQ451098

[a] The carbon source used in enrichment of BTX degrading bacterium

Pseudomonas stuzeri 11C2 (AJ270452) (99%). LJ2 and LE2 were *Rhodococcus* bacteria, degrading toluene and xylene, respectively; LJ2 was closest to *Rhodococcus pyridinivorans* strain R04 (AF459741) (99%), while LE2 was closest to *Rhodococcus rhodochrous* strain DSM43274T (X80624) (99%). Another toluene degrader LJ8 was closest to *Exiguobacterium gaetbuli* strain TF-16 (AY594264) (99%). The only benzene degrader, LB1 showed to be *Bacillus subtilis* strain MO5 (AY553098) (99%). Excepting *Exiguobacterium* genus, bacteria of other genera have been previously reported as BTEX degraders (Arenghi et al. 2001; Kim et al. 2002; Hendrickx et al. 2006b; Wang and Shao 2006).

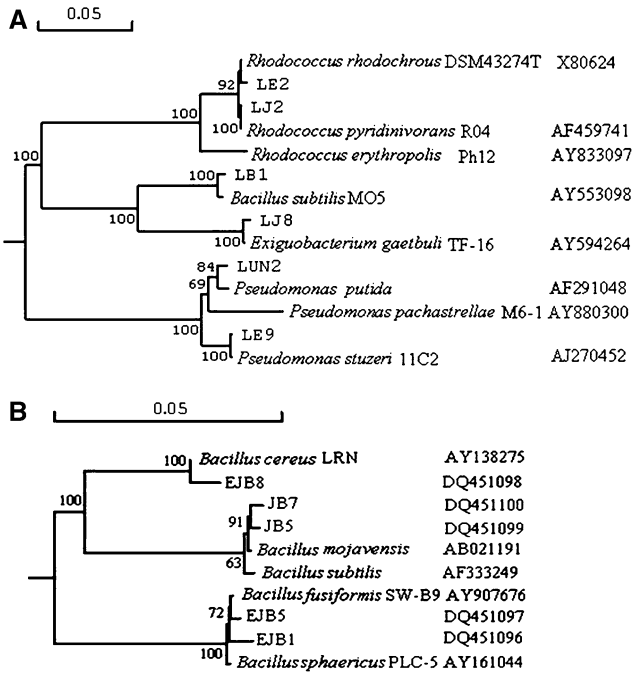

Fig. 1 Phylogenetic trees based on 16S rDNA sequences of BTX degrading bacteria **a** isolated from surface water of Xiamen Ferry Dock **b** isolated from deep-sea sediments of the west Pacific sediment. Bootstrap values (expressed as percentages of 1,000 replications) are shown at *branch points*. *Bar* 0.05 nucleotide substitution rate (Knuc) units

In contrast to the diversity of coastal bacteria, all isolates from Pacific deep-sea sediment belonged to *Bacillus* (Fig. 1b). Two toluene degraders JB5 and JB7 showed to be phylogenetically closest to a deep-sea bacterium *Bacillus mojavensis* (AB021191) (99%). Although they showed 99% homology in 16S rDNA, the morphology on plate of the two bacteria was completely different. Similarly, two xylene degraders EJB1 and EJB5, were close to each other and both shared 99% homology to *Bacillus fusiformis* SW-89 (AY907676) (99%); they varied in colony color and shape on plate. Another xylene degrader EJB8 was closest to *Bacillus cereus* LRN (AY138275) (99 %).

All the results of bacteria characterization are summed up in Table 1.

Characteristics of the isolates: optimal temperature, salinity and pH

Each bacterium was inoculated into MM medium containing 0.1% benzene, toluene or xylene, growing at 4, 18, 28 and 37°C at 150 rpm. At an interval of about 8 h, cell density was measured at OD_{600}. Results showed that 28°C was the optimal growth temperature for all bacteria from the Dock water; this is in agreement with the in situ temperature. The bacteria from Pacific Ocean sediment showed the best growth at 18°C, and good growth at 28 and 37°C, but only a slight growth at 4°C.

Salinity tests showed that all bacteria of both environments tolerate up to 6% salinity, with 3% as the optimal. In

addition, all bacteria showed a preference to alkaline pH, ranging from pH 7 to 10. In contrast, most previously reported BTEX-degrading bacteria grow in neutral pH (Margesin and Schinner 2001; Xi et al. 2005; Zhang et al. 2005).

Tolerance to organic solvents

Each bacterium from deep-sea sediment was inoculated in a flask containing 30 ml HLB medium of different concentrations of toluene or xylene from 0 to 90% (v/v), with 5, 10, 15, 20, 30, 50 and 70% as in-betweens. After 5 days incubation at 150 rpm, OD_{600} of culture was measured and cell viability was examined. As a result, JB5 grew in presence of 15% (v/v) toluene, EJB1, EJB5 and EJB8 grew in presence of 5% (v/v) xylene. In the case of JB7, the cultures of toluene treatments showed values of OD_{600}. However, when toluene was above 10%, the cells suffered a severe morphological aberration, narrowed, irregular and no spore was formed; what's more, viability was lost as examined on plate.

Those from dock water were not examined in a wide range of solvent concentration as above. But all the six isolates showed tolerance against ethylbenzene and *p*-xylene with the highest tested concentration of 20% (v/v), and LJ2, LJ8, LE2 and LE9 showed tolerance to 10% (v/v) toluene; LB1, LE9 and LJ8 could tolerate 5% (v/v) benzene.

Degradation range to aromatic compounds

With each of the tested aromatic compounds as the sole carbon source, the degradation range of each isolate was examined. Among the deep-sea isolates, EJB1 showed a broad degradation range, it was initially isolated from xylene enrichment, but grew on benzene and toluene as well (Table 2). JB5 obtained from toluene enrichment grew with benzene as well. Others only degraded the substrates used originally for enrichment. EJB1, EJB5 and EJB8 obtained from xylene enrichment grew only with xylene and JB7 initially enriched with toluene grew only with toluene (Table 2).

Similarly, the bacteria from dock water were also tested for their degrading range to BTEX (Table 2). As a result, LB1, LE2 and LE9 degraded all the four compounds and better growth was observed with ethylbenzene and *p*-xylene. Benzene degradation was only observed with plate cultivation, not in liquid medium of 20% (v/v) benzene. Another broad range bacterium was LJ8, which grew with ethylbenzene and *p*-xylene in addition to toluene; while a slight growth with benzene was observed as well, the growth with benzene was further confirmed by plate supplied with benzene vapor. LJ2 could grow with toluene and ethylbenzene, but it could not grow with benzene, while with *p*-xylene only a very weak growth occurred. LUN2 grew vigorously in liquid medium with *p*-xylene (20%, v/v), it was also confirmed as a toluene degrader on plate.

Table 2 BTEX degradation range of bacteria from nearshore water and deep-sea sediment

Isolates	Benzene	Toluene	Ethylbenzene	*p*-Xylene
LB1	+	+	++	++
LE2	+	+	++	++
LE9	+	+	++	++
LJ2	–	+	+	+
LJ8	+	+	+	++
LUN2	–	+	+	++
JB5	+	+	/	–
JB7	–	++	/	–
EJB1	+	+	/	++
EJB5	–	–	/	+
EJB8	–	–	/	+

– no growth, + growth positive, ++ vigorous growth, / not examined

Oxygenase genes involved in BTX degradation

From all six isolates of the dock water, a fragment of benzene dioxygenase was PCR-cloned with a pair of primers of Ben-F and Ben-R, specially designed for detecting the gene encoding alpha subunit of benzene dioxygenase (Fig. 2a). Sequence analysis showed that the obtained fragments of benzene dioxygenase (358 bp) shared a complete identity, and had 96% homology with those of strains isolated from benzene polluted soil (unpublished, available in GenBank with accession No. as J60924, AJ609526-29, AJ609532, AJ609536 and AJ609537), but relatively far from the genes from *Pseudomonas putida* (Irie et al. 1987) and *Pseudomonas aeruginosa* (Nishimura et al. 1993) (Fig. 2a). Meanwhile, only from xylene degrading isolates LE9 (*P. stuzeri*, 99%) and LUN2 (*P. putida*, 99%), a fragment of toluene dioxygenase (753 bp) was detected with a pair of primers (TODC1-F and TODC1-R) (Fig. 2b), sharing 99% similarity with that from a toluene degrading *P. putida* (Y18245) (Mosqueda et al. 1999). This indicated that the two xylene degraders possibly degrade toluene as well, and confirmed by substrate test above. In contrast, neither benzene nor toluene dioxygenase gene could be detected from the deep-sea isolates, as well as xylene monoxygenase gene.

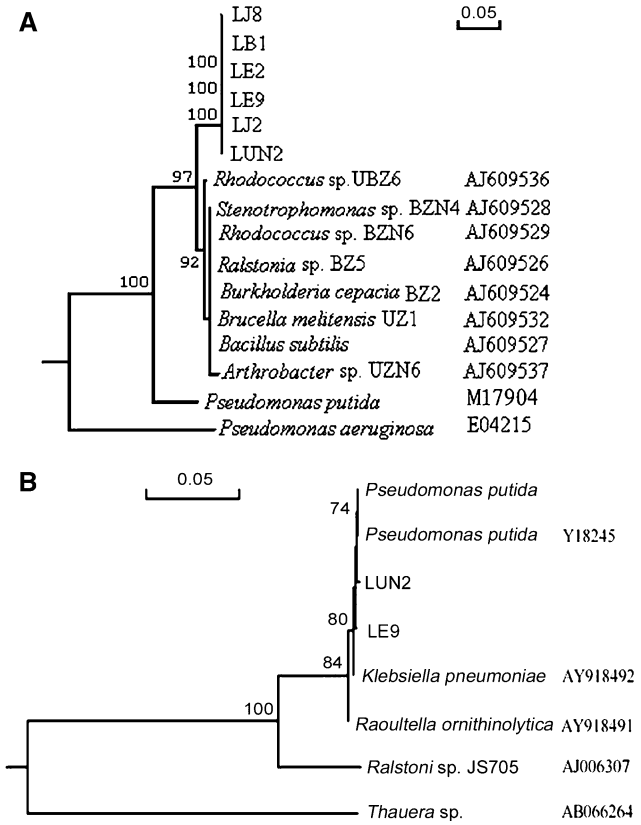

Fig. 2 The phylogenetic trees of **a** bacterial benzene dioxygenase genes and **b** toluene dioxygenase genes to show to position of the genes cloned from six nearshore bacteria. Gene accession No. of GenBank is listed at *right*. Bootstrap values (expressed as percentages of 1,000 replications) are shown at *branch points*. *Bar* 0.05 nucleotide substitution rate (Knuc) units

Discussion

It is well known that volatile hydrocarbons, such as benzene, toluene and xylene, which are abundant in many refined oils and toxic to many kinds of life. In this report, BTX degrading bacteria in nearshore and deep-sea environments were investigated. Among the previously reported BTX degrading bacteria, *Pseudomonas*, *Acinetobacter* and *Rhodococcus* are most important, which are frequently found as BTX degraders in polluted soils and sewages (Greene et al. 2000; Hendrickx et al. 2006a; Wang and Shao 2006). In this report, we found that in nearshore surface water, degrading bacteria showed a relatively wide diversity, including species of *Pseudomonas*, *Rhodococcus*, *Exiguobacterium* and *Bacillus*. But in Pacific Ocean sediment, *Bacillus* absolutely dominated the BTX degrading population. The dominance of Gram-positive bacteria in two environments in this report goes against the general recognition that Gram-negative bacteria are the main group of organic pollutant degraders (MacNaughton et al. 1999).

From all isolates of the ferry dock water including the toluene or xylene enriched bacteria, a homological benzene dioxygenase gene was confirmed to be present, whereas two similar toluene dioxygenase genes were detected only from two xylene degrading isolates, but the gene encoding toluene/xylene monoxygenase was not detected from all isolates. Lateral transfer of benzene degrading genes among species in situ of Xiamen nearshore environment was strongly supported. Differently, *Bacillus* species from deep sea were negative in PCR detection of the above genes with all the primers described in "Materials and methods". Whether the deep-sea isolates own a unique metabolic pathway remained unclear.

Deep sea is remote from pollution of hydrocarbons like oil spill, yet it was regarded as an ideal place to isolate such bacteria. Kato et al. (1996) reported that organic-solvent-tolerant bacteria were over 100 times more abundant in deep-sea mud samples than in terrestrial soils. As a pioneer study, Inoue and Horikoshi (1989) reported a bacterium *Pseudomonas* that thrived in high concentrations of toluene (30–50%, v/v). Latter, a benzene tolerant *Bacillus* sp. was isolated from deep sea (Moriya and Horikoshi 1993). In our report, all the isolates of both coastal and deep-sea environments showed a certain degree of tolerance to the solvents. Most coastal isolates can grow in presence of 20% solvents, while the tolerance of deep-sea isolates was below 15%. These concentrations are lethal and extreme environment to normal bacteria. In the case of two previously reported BTEX degraders of *Pseudomonas*, cell growth was completely inhibited at 500 mg l^{-1} of benzene, 600 mg l^{-1} of *o*-xylene and 1,000 mg l^{-1} of toluene (Shim et al. 2005). And in the case of an algae *Skeletonema* sp., growth rate was significantly reduced by benzene at concentrations above 10–20 ppm (Atkinson et al. 1976).

Worth to note, cell viability examination is crucial and necessary to detect bacterial tolerance because pseudo-growth occurred in treatments of high solvent concentrations. The turbidity of the culture was not the direct result of raised cell concentration. During the investigation of tolerance of deep-sea isolates, JB7 was regarded of high-tolerance, judged from the high OD_{600} of the cultures even to 90% toluene. Actually, the cell lost its viability and suffered a severe morphological change when toluene concentration was above 15%. But for an unknown reason, a high-turbidity was resulted.

Summarily, BTX degrading bacteria were confirmed to be present in both nearshore and deep-sea environments. Most of them showed a wide range of degradation. Undoubtedly, they play a role in attenuation of these toxic compounds in marine environments. Their adaptability to saline conditions and high-concentration of organic solvent make them unique in bioremediation.

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