J Ind Microbiol Biotechnol (2010) 37:927–934
DOI 10.1007/s10295-010-0740-7

ORIGINAL PAPER

Versatile aromatic compound-degrading capacity and microdiversity of *Thauera* strains isolated from a coking wastewater treatment bioreactor

Yuejian Mao · Xiaojun Zhang · Xi Xia · Huihui Zhong · Liping Zhao

Received: 11 April 2010 / Accepted: 3 May 2010 / Published online: 21 May 2010
© Society for Industrial Microbiology 2010

Abstract Bacteria of the *Thauera* genus have been described as important aromatic compound degraders and have attracted increased attention. In this study, three *Thauera* strains (Q4, Q20-C, and 3–35) were isolated from a coking wastewater treatment plant (WWTP) with a high abundance of *Thauera*. The 16S rRNA, nitrite reductase, and phenol hydroxylase (LmPH) genes and pollutant-degrading capacity of these strains were characterized and compared. Their 16S rRNA gene sequences were identical, but the genomic structures differed, as demonstrated by distinct enterobacterial repetitive intergenic consensus sequence PCR profiles with a similarity of less than 0.65. The analysis of degradation of coking wastewater by these strains showed that most of the main organic pollutants—phenol, methylphenol, and indole, but not quinoline—were degraded under aerobic conditions. These strains contained different LmPHs genes and showed different phenol degradation rates (Q4 > 3–35 > Q20-C). The presence of a microdiversity of *Thauera* spp. implies the existence of various finely differentiated niches in the industrial WWTP. The capacity of the *Thauera* strains to degrade a wide spectrum of aromatic compounds suggests their potential in bioremediation applications targeting aromatic pollutant-containing wastewater.

Keywords *Thauera* · Aromatic compound · Degradation · Microdiversity

Y. Mao · X. Zhang · X. Xia · H. Zhong · L. Zhao
Key Laboratory of Microbial Metabolism, Ministry of Education, School of Life Science and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China

Present Address:
X. Xia
Lanzhou University, Lanzhou, Gansu, China

X. Zhang (✉)
School of Life Science and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China
e-mail: xjzhangsjtu@gmail.com

Introduction

Bacteria of the *Thauera* genus, of the family Rhodocyclaceae of the beta-subclass of Proteobacteria, show versatile aromatic compound-degrading capacity [4, 13, 17–20]. The aromatics, such as phenol and cresol, were firstly transformed into a central intermediate benzoyl-CoA by *Thauera* under anaerobic conditions through different peripheral pathways, and then degraded through the same central benzoyl-CoA pathway [7]. *Thauera* bacteria have been recognized as functionally important populations in several wastewater treatment plants (WWTPs) [9, 10, 21, 22]. In our previous work, we found that *Thauera* spp. formed a predominant population (11.46%) in a full-scale coking WWTP [12], in which the main organic pollutants were aromatic compounds [23]. The fluctuation of chemical oxidation demand (COD) removal function of the WWTP was closely related to the structural shifts of *Thauera* bacteria in the bioreactor, which suggests that these organisms might play important roles in this coking WWTP. Moreover, sequencing analysis of the 16S rRNA gene revealed that the predominant *Thauera* bacteria in this WWTP was different from those reported by other researchers [12]. Thus, isolation of the *Thauera* bacteria from this WWTP could essentially be helpful to further improve our understanding of their functions and ecological attributes. However, none of them was purified after many trials using the conventional isolation methods that

were modified from previous studies [13, 17, 18]. The difficulty in purifying *Thauera* bacteria with conventional methods was probably due to the great variety of their physiological traits. In total, around 50 *Thauera* strains have been isolated worldwide, most were enriched or isolated under denitrification conditions, and all the characterized strains were known to be facultative denitrifiers. However, these strains were not bound to be the predominant ones in the community. As a result of the high diversity and varied physiological traits of *Thauera* bacteria [13, 20], the isolation methods used in previous studies may not be suitable for isolating the bacteria living in other different environments, such as the coking WWTP. Therefore, application of a new strategy is essential to allow isolation of the *Thauera* not previously isolated, thus enriching the available resource of versatile aromatic compound-degrading bacteria.

Here we report isolation of three *Thauera* strains with identical 16S rRNA genes from a full-scale coking WWTP by using a sequence-guided approach [11]. The aromatic pollutant-degrading capacities and related functional genes (LmPHs and *nirS*) of the strains were characterized and compared.

Materials and methods

Isolates and culturing conditions

The biofilms, where the *Thauera* strains were isolated, were collected from the anoxic tank (named A2) of a full-scale anaerobic-anoxic-aerobic (A1-A2-O) WWTP, located in Shanghai, China. The influent coking wastewater of the A2 tank was collected from this WWTP and used as the cultivation medium for pollutant degradation experiments after sterilization by successive filtering through 0.45-μm and 0.22-μm membrane filters (Pall) and adjusting to pH 7.5. For denitrification testing, a modified basic chemical medium (1 L, pH 7.5) was used, which contained 0.45 g NH_4Cl, 0.05 g K_2SO_4, 0.1 g $MgCl_2{\cdot}6H_2O$, 0.025 g $CaCl_2{\cdot}2H_2O$, 0.04 g $NaHCO_3$, 0.2 g NaAc, 0.5 g peptone, and 0.3 g meat extract (Fluka), supplemented with 0.1 M phosphate buffer (Na_2HPO_4 and NaH_2PO_4) and 2 mM nitrate/nitrite. The changes in concentration of nitrate and nitrite in the media were measured by using ion chromatography to determine their denitrification ability. *Thauera linaloolentis* (DSM12138), which showed aerobic growth in this medium, was used as a positive control. Peptone and meat extract were substituted by different concentrations of phenol (45 and 160 mg/l) for phenol degradation measurement.

A 8-ml aliquot of medium was distributed into each autoclaved 34-ml serum flask (containing a magnetic stirring bar). A 1-ml aliquot (0.9 O.D.) of culture with the strain isolated from the biofilm of a coking WWTP and prepared under aerobic conditions was inoculated into each flask. For aerobic incubation, flasks were covered with an aluminum membrane. For anaerobic incubation, 2 mM nitrate was added and flasks were tightly sealed with rubber septa (rubber stoppers, N 20, light gray, Macherey-Nagel, Germany) and aluminum caps (CNW, Germany). Anaerobic conditions were established by flushing the flask with N_2 for 5 min. The microbiological Anaerotest® (Merck KGaA, Germany) was used to confirm the anaerobic conditions. Flasks were incubated at 28°C with stirring at 500 rpm for 4 days. Samples were collected and stored at −20°C. All these cultivation experiments were repeated three times.

Chemical analysis

Samples were centrifuged at 10,000×*g* for 10 min to remove the cells. Organic compounds were extracted from 1 ml of the supernatant with 0.1 ml CH_2Cl_2 (HPLC grade). A total of 1 μl of extracted content was analyzed by using a Shimadzu GC-2010 equipped with a DB-5 column (30-m length, 0.25-mm inner diameter) and a flame ionization detector, using the following method: the injector temperature was 280°C; the column of the GC was retained at 70°C for 3 min, and then increased to 280°C with an increment of 5°C/min; the temperature for the MS ion source was 200°C; and electron energy was 70 eV [23].

DNA extraction and PCR analysis

Genomic DNA was extracted from the collected bacterial cells by using the bead-beating method [24]. Primers E1 (5′-ATGTAAGCTCCTGGGGATTCAC-3′) and E2 (5′-AAGTAAGTGACTGGGGTGAGCG-3′) were used in enterobacterial repetitive intergenic consensus sequence (ERIC)-PCR [6]. The 25-μl PCR reaction mixture contained 2.5 U Taq DNA polymerase (Promega Co., USA), 2.5 μl 10× buffer, 1.5 mM $MgCl_2$, 200 μM each deoxynucleoside triphosphate (dNTP), 10 pmol of each primer, and 80 ng total DNA, and the reaction was performed in an automated thermocycler (PCR Sprint, Thermo Electron, Corp., UK) using the following program: 7-min pre-denaturation at 95°C; 30 cycles of denaturation at 95°C for 30 s; annealing at 50°C for 1 min; and extension at 65°C for 8 min; followed by a final extension at 65°C for 16 min. The concentration of the PCR product was determined by using a DyNA Quant 200 fluorometer (Pharmacia, San Francisco, CA, USA). A total of 200 ng of the PCR product was then resolved on a 1.2% (wt/vol) agarose gel. The gel was stained with ethidium bromide and photographed by using a UVI gel documentation system

(UVItec, Cambridge, UK). The migration distance and intensity of the ERIC-PCR bands were analyzed by using Quantity One (version 4.4.0, Bio-Rad, California) according to the manual. The band intensity data were normalized into percentages for statistical analysis. Cluster analysis was performed by using PAST software (Palaeontological Statistics, ver. 1.58) with a Dice coefficient.

Partial nitrite reductase genes (*nirS* ~ 890 bp and *nirK* ~ 514 bp) were amplified by using the primer pairs nirS-1F (5′-CCTAYTGGCCGCCRCART-3′) and nirS-6R (5′-CGTTGAACTTRCCGGT-3′), and nirK-1F (5′-GGMA TGGTKCCSTGGCA-3′) and nirK-5R (5′-GCCTCGATC AGRTTRTGG-3′), respectively, using a program of 94°C for 5 min; 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min; and 72°C for 7 min [3]. Partial LmPH (the largest subunit of the bacterial multicomponent phenol hydroxylase, ~620 bp) gene was amplified by using the primer pairs pheUf (5′-CCAGGSBGARAARGAGAR GAARCT-3′) and pheUr (5′-CGGWARCCGCGCCAGA ACCA-3′), with a program consisting of 94°C for 10 min; 5 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 1 min; 5 cycles of 94°C for 1 min, 57°C for 1 min, 72°C for 1 min; 25 cycles of 94°C for 1 min, 56°C for 1 min, 72°C for 1 min; and then 72°C for 10 min [5]. The 25-μl PCR reaction mixture contained 1 U Taq DNA polymerase (Promega Co., USA), 1× PCR buffer (Mg^{2+} free), 2 mM $MgCl_2$, 10 pmol of each primer, 200 μM each dNTP, and 20 ng template DNA.

Sequencing analysis

PCR products were analyzed by using agarose gel electrophoresis. Target bands were excised from the gel and purified by using a DNA gel extraction kit (V-gene Biotechnology Limited, Hangzhou, China). Purified PCR products were ligated to a pGEM-T easy vector (Promega Co., USA) and transformed to *E. coli* DH10B competent cells. Clones were randomly selected and sequenced by Sangon (Shanghai, China). The obtained sequences were then analyzed by Blastn and Blastx against the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/BLAST/).

The nucleotide sequences of *nirS* and LmPH genes have been deposited in the GenBank database with the accession numbers GU566032–GU566035.

Results and discussion

Isolation of *Thauera* strains

After several failed attempts at isolating *Thauera* bacteria from the full-scale coking WWTP with conventional isolation methods, a new isolation strategy using *Thauera*-specific 16S rRNA gene as the indicator was employed to assist the isolation [11]. Six types of media (nutrient broth, 10 times diluted nutrient broth, sterile wastewater, basic

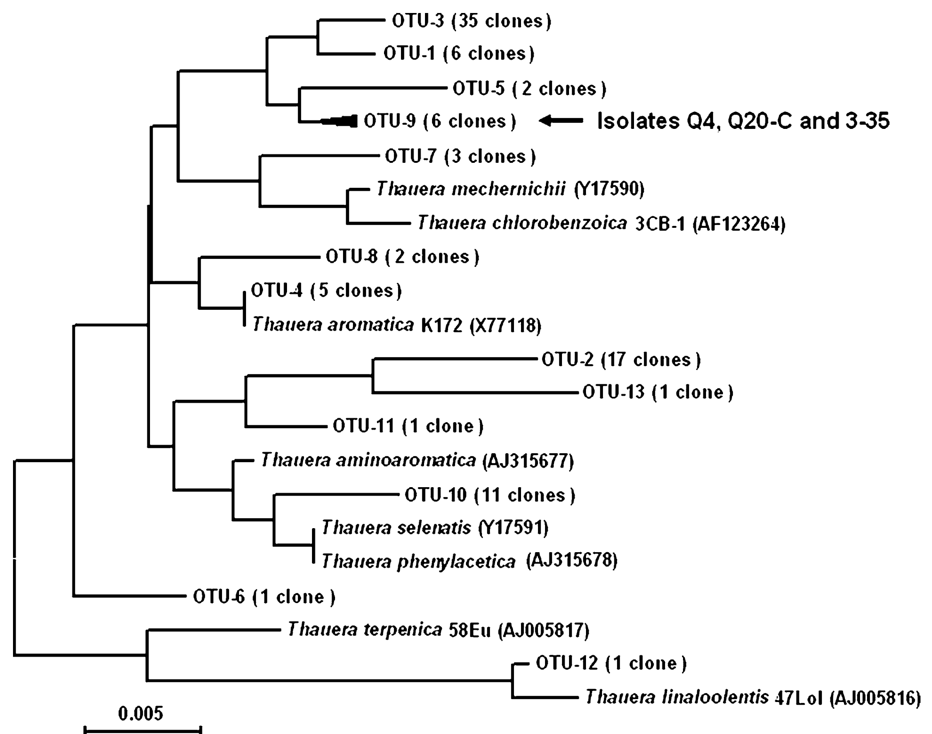

Fig. 1 Phylogenetic analysis of the *Thauera* isolates. OTU1–13 are the operational taxonomic units (OTUs) of a *Thauera*-specific 16S rRNA gene clone library of the biofilm sample from the same place where those *Thauera* strains were isolated [12]

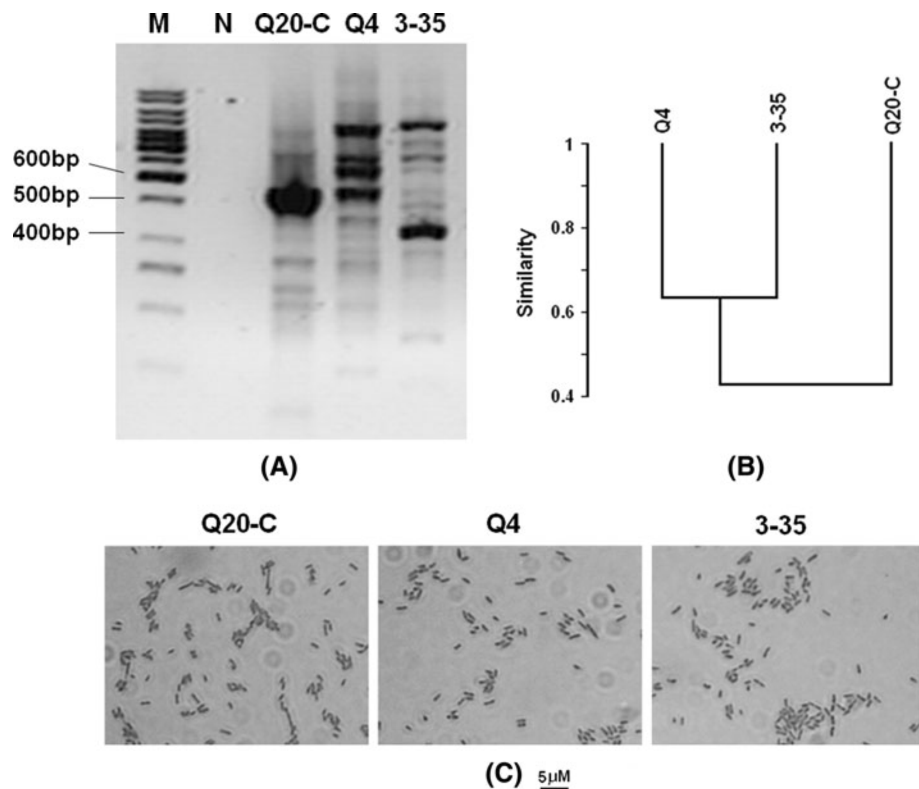

Fig. 2 **a** Different ERIC-PCR fingerprints of the *Thauera* isolates with identical 16S rRNA genes (*M* DNA ladder, *N* negative control). **b** Cluster analysis of the ERIC-PCR profile using the Dice coefficient. **c** Microscopy photographs (×1,000) of pure cultures of strains (note the morphology of these strains was very similar)

mineral medium with NaAc/phenol/quinoline) were used both in aerobic and anaerobic conditions for the cultivation. The abilities of these media in culturing *Thauera* spp. were assessed by using a *Thauera*-specific PCR-denaturing gradient gel electrophoresis (DGGE) method to analyze the diversity of colonies growing on the media. The media 1/10 NB (10 times diluted nutrient broth) and MMQ (mineral medium supplemented with quinoline), which supports a higher diversity of *Thauera* spp. and fewer colonies respectively, were selected to isolate *Thauera* bacteria. More than 320 colonies that grew on these two media were then screened by using *Thauera*-specific PCR, which yielded five colonies with positive signal. Their homogeneity was then checked by using 16S rRNA gene (V3 region) PCR-DGGE. The PCR-DGGE of 16S rRNA gene universal V3 region showed that three colonies exhibited multiple bands, which suggests these colonies had failed to be purified even after several rounds of streaking on the medium from which they originated. However, after being streaked on other selective media (mineral medium supplemented with acetate, phenol, or quinoline) and tracked using *Thauera*-specific PCR and DGGE, *Thauera* spp. in a positive colony, Q20, were enriched on the basic mineral medium with phenol and finally purified after several rounds of streaking. In total, three strains (Q4, Q20-C, and 3–35) were purified from the coking WWTP sample.

Thauera spp. have been reported to be an important population with a high abundance in the ecosystem [10]. However, *Thauera* bacteria were difficult to isolate in pure culture probably due to their low growth rate during the isolation and are prone to be outgrown by other bacteria. *Thauera*-specific PCR and PCR-DGGE were used to track the *Thauera* bacteria during the isolation for screening the optimum isolation conditions and selecting of *Thauera* sequence-containing colonies. This strategy largely increased the isolation efficiency, and should also have great advantages for isolation of the other bacteria which were difficult to isolate by conventional methods.

Phylogenetic analysis of *Thauera* isolates

Sequencing analysis of the three isolates revealed that they had identical 16S rRNA genes (accession numbers in GenBank EU850614–EU850616), and they were assigned to the *Thauera* genus based on Ribosomal Database Project classifiers (http://rdp.cme.msu.edu/classifier/classifier.jsp). These isolates shared 100% similarity with the 16S rRNA gene of *Thauera* sp. PIV-1 (AJ505850), which can use pivalate under denitrification conditions [14]. However, their closest type strain was *Thauera chlorobenzoica* 3CB-1 (AF123264), with which they shared 97% 16S rRNA gene similarity.

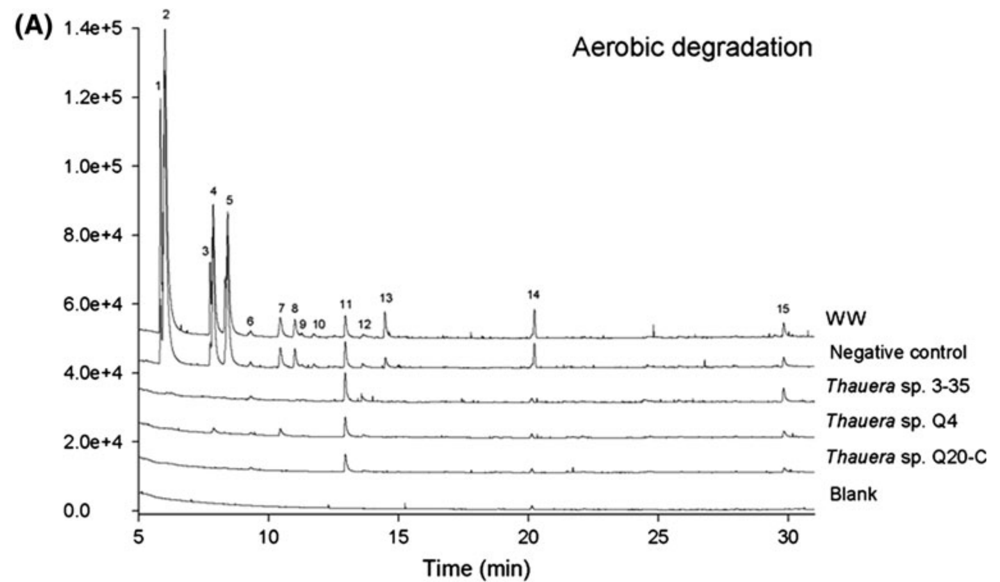

Fig. 3 GC-MS analysis of organic compounds in the coking wastewater. WW, the GC profile of coking wastewater that was used as media in the cultivation; *Thauera* sp. 3–35, Q4 and Q20-C, the GC profiles of coking wastewater after 4-day aerobic incubation with these *Thauera* strains; negative control, the coking wastewater incubated under the same conditions but without inoculation with any bacteria; blank, the blank control in GC analysis. **a** The relative proportion of organic pollutants in the coking wastewater (before incubation), calculated based on the peak area. **b** The degrading capacity of these strains for each peak after 4-day aerobic incubation: +, significantly removed; −, no degradation; Δ, slightly decreased

(B)

Peaks	Pollutants	Identity (%)	[a]Proportion in WW (%)	[b]Degradation 3-35	Q4	Q20-C
1	Phenol	95	52.5	+	+	+
2				+	+	+
3	Cresol	97	33.1	+	+	+
4				+	+	+
5				+	+	+
6	Dimethylphenol	90 - 97	5.7	Δ	Δ	Δ
7				+	+	+
8				+	+	+
9				+	+	+
10				+	+	+
11	Quinoline	96	2.7	-	-	-
12	Ethylmethylphenol	76	0.7	+	+	+
13	Indole	96	2.3	+	+	+
14	Butylated hydroxytoluene	95	1.7	+	+	+
15	Dibutyl phthalate	94	1.3	-	Δ	Δ

Previous investigation revealed quite high diversity of *Thauera* spp. in this coking WWTP by using a *Thauera*-specific clone library [12]. A 16S rRNA gene phylogenetic tree was built for these isolates and the clones of the *Thauera*-specific clone library for the same WWTP, and also all eight know *Thauera* species (Fig. 1). The 16S rRNA genes of these isolates were clustered to OTU-9, which contains 6 clones. The cluster where the isolates located contains the most predominant *Thauera* species in this full-scale coking WWTP, which accounted around 60% of the total clones in the library.

Different genomic fingerprints of the *Thauera* isolates

ERIC-PCR is an efficient genomic fingerprinting technique that has been widely used to differentiate strains with close or even identical 16S rRNA genes [8, 15]. Figure 2a shows the distinct ERIC-PCR profiles of strains Q4, Q20-C, and 3–35. The differences among the fingerprinting patterns were evaluated by cluster analysis using the Dice coefficient method (Fig. 2b), and their genomic fingerprinting similarities proved to be less than 0.65. This result demonstrated the wide variation of genomic structure of *Thauera* strains with identical 16S rRNA genes isolated from the same habitat. The variation is probably attributable to the heterogeneity of the physicochemical conditions within the biofilm [16], where the strains occupy different niches and face different selective pressures. This finding suggests that the diversity of the *Thauera* population in the environment, usually evaluated using the 16S rRNA gene clone library method, may be significantly underestimated.

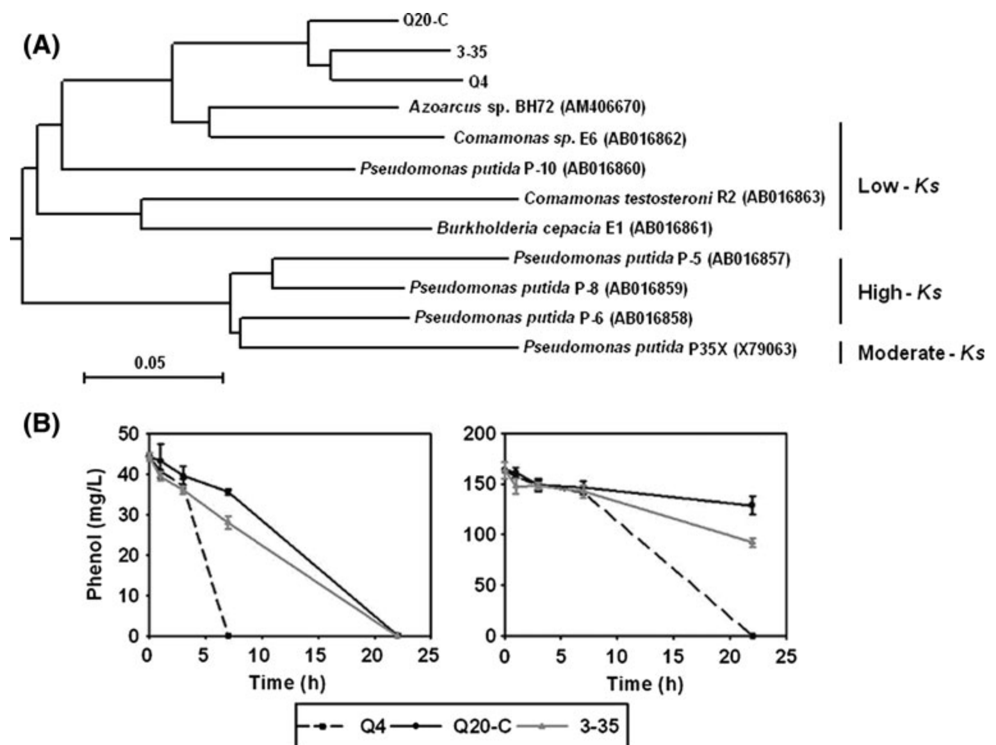

Fig. 4 **a** Phylogenetic analysis of the largest subunit of the bacterial multicomponent phenol hydroxylase (LmPH) of *Thauera* isolates. **b** Phenol degradation curves of these isolates at two different starting concentration (45 and 160 mg/l) under aerobic conditions at 28°C. Each experiment was repeated three times

Aromatic pollutant-degrading capacity of the *Thauera* isolates

The aromatic compound-degrading capacity of isolated bacterial strains was evaluated under both aerobic and anoxic conditions. To understand their functions in the native environment, the medium used was the coking wastewater, which was collected from the bioreactor where the isolates originated and sterilized by filtration. Gas chromatography–mass spectrometry (GC-MS) analysis of the coking wastewater revealed a complicated composition involving 15 components (Fig. 3). Phenol and cresol were the most predominant organic pollutants in the wastewater. After a 4-day incubation under aerobic conditions, almost all of the organic pollutants in the coking wastewater, except quinoline, were completely degraded by any of the three isolates. This result demonstrated the capacity of these *Thauera* strains for aerobic degradation of a wide spectrum of aromatic compounds. However, the strains degraded nothing under anoxic conditions. To the best of our knowledge, most of the known *Thauera* species have more versatile aromatic compound-degrading ability under denitrification conditions compared to aerobic conditions, including *T. aromatica* and *T. aminoaromatica* [1, 13]. Only *T. phenylacetica* is known to use phenol under aerobic conditions [13].

Denitrification capacity of the *Thauera* isolates

All of the *Thauera* strains characterized prior to this work were known to be facultative denitrifiers. However, these isolates did not perform denitrification either in the coking wastewater or in the several other media in which they can grow aerobically. The nitrite reductase genes (*nirS*/*nirK*) of these strains were analyzed by PCR with primer pairs nirS-1F/nirS-6R and nirK-1F/nirK-5R [3]. Only strain Q20-C showed a *nirS* gene-positive PCR signal. The PCR product of this strain was purified and cloned, and sequencing analysis showed that its closest sequence was the *nirS* gene of *Thauera aromatica* T1, with which it shared 90% nucleotide sequence identity and 95% translated protein sequence similarity.

Phenol-degrading efficiency and the LmPHs gene of the *Thauera* isolates

Phenol is the most predominant organic pollutant in coking wastewater. The degrading efficiency of these three *Thauera* strains for phenol was evaluated under aerobic conditions (Fig. 4b). With an initial concentration of 45 mg/l, 100, 19.7, and 36.8% (average value of triplicate tests) of the phenol was removed by strains Q4, Q20-C, and 3–35, respectively, after a 7-h incubation in the mineral medium at 28°C. With an initial concentration of 160 mg/l,

Table 1 Comparison of the three isolates with known *Thauera* species

	Q4	Q20-C	3–35	*T. chlorobenzoica* 3CB-1^T	*T. aromatica* K172^T	*T. linaloolentis* 47Lol^T	*T. terpenica* 58Eu^T	*T. mechernichensis* TL1^T	*T. selenatis* AX^T	*T. aminoaromatica* S2^T	*T. phenylacetica* B4P^T
Denitrification	−	−	−	+	+	+	+	+	+	+	+
nirS	−	+	−	+	+	+	+	+	+	+	++[a]
[b]Phenol	+/−	+/−	+/−	−/−	−/+	ND	ND	ND	ND	ND/+	+/+
[b]Cresol	+/−	+/−	+/−	ND	−/+	ND	ND	ND	ND	ND/+	−/+
[b]Indole	+/−	+/−	+/−	ND	−/+	ND	ND	ND	ND	−/+	−/+

ND not determined

[a] *T. phenylacetica* contains two types of *nirS* gene

[b] The aromatic compound-degrading capacities were evaluated under aerobic/anoxic conditions

100, 21.2, and 43.6% of the phenol was removed by strains Q4, Q20-C, and 3–35, respectively, after 22 h. The phenol degradation rates of these strains differed from each other, in the order Q4 > 3–35 > Q20-C.

The LmPH gene, which encodes the key enzyme for aerobic metabolism of phenol, was amplified from each strain by using primers pheUf/pheUr and then sequenced [5]. The LmPH genes of Q4, 3–35, and Q20-C also differed, with Q20-C exhibiting the lowest similarity with the others (Fig. 4a), which has lower phenol degradation rate. This result showed the consistency between the phenol degradation activity and LmPH gene sequence. Phylogenetic analysis with related LmPH genes in GenBank suggested that the three isolates may belong to low-*Ks*-type phenol-degrading bacteria (Fig. 4a). The nearest neighbor of these strains in terms of LmPH genes is *Azoarcus* sp. BH72 (AM406670), but it has less than 87% sequence similarity with the potentially new type of LmPH gene of these three *Thauera* isolates.

In conclusion, genetic and physiological divergence was observed among these three phylogenetically identical *Thauera* strains, which originated from the same coking WWTP. Previous studies have revealed microdiversity among phylogenetically identical strains; for example, Jaspers and Overmann [8] found that 11 *Brevundimonas alba* strains with identical 16S rRNA genes, isolated from the same freshwater sample, exhibited highly divergent genomes and ecophysiologies. To the best of our knowledge, only two strains (*Thauera aromatica* SP and LG356) in the *Thauera* genus have been reported to share identical 16S rRNA genes [13], but their G + C content and substrate-using characteristics differed; SP can use phenol, toluene, and *p*-cresol, while LG356 cannot use any of these [13]. However, these differences were probably the result of their different original environments with different selection pressures [2]. The biological significance of the microdiversity of those three isolates, which represent a functionally important population from the same bioreactor, requires further investigation. Although these three isolates exhibited microdiversity among themselves, the obvious differences in the 16S rRNA genes and physiological attributes between these isolates and the known *Thauera* species (Table 1) suggest that they may represent a new *Thauera* species with a high ability to degrade a wide spectrum of aromatic compounds under aerobic conditions. Sequencing the whole genomes of these isolates will be a promising next step to obtain a deeper understanding of these versatile aromatic compound degraders. Continuing efforts also are focused on isolating more *Thauera* strains from the coking WWTP.

Acknowledgments This research was financially supported by the National Natural Science Foundation of China (NSFC 20677041), and

National “863” High-tech R&D Program (2007AA021301), the Shanghai Leading Academic Discipline Project (B203), and the project 05SR07107 sponsored by Shanghai-Rhone bilateral collaboration research.

References

1. Anders HJ, Kaetzke A, Kampfer P, Ludwig W, Fuchs G (1995) Taxonomic position of aromatic-degrading denitrifying pseudomonad strains K 172 and KB 740 and their description as new members of the genera *Thauera*, as *Thauera aromatica* sp. nov., and *Azoarcus*, as *Azoarcus evansii* sp. nov., respectively, members of the beta subclass of the Proteobacteria. Int J Syst Bacteriol 45:327–333
2. Beiko RG, Harlow TJ, Ragan MA (2005) Highways of gene sharing in prokaryotes. Proc Natl Acad Sci USA 102:14332–14337
3. Braker G, Fesefeldt A, Witzel KP (1998) Development of PCR primer systems for amplification of nitrite reductase genes (*nirK* and *nirS*) to detect denitrifying bacteria in environmental samples. Appl Environ Microbiol 64:3769–3775
4. Foss S, Harder J (1998) *Thauera linaloolentis* sp. nov. and *Thauera terpenica* sp. nov., isolated on oxygen-containing monoterpenes (linalool, menthol, and eucalyptol) nitrate. Syst Appl Microbiol 21:365–373
5. Futamata H, Harayama S, Watanabe K (2001) Group-specific monitoring of phenol hydroxylase genes for a functional assessment of phenol-stimulated trichloroethylene bioremediation. Appl Environ Microbiol 67:4671–4677
6. Gillings M, Holley M (1997) Repetitive element PCR fingerprinting (rep-PCR) using enterobacterial repetitive intergenic consensus (ERIC) primers is not necessarily directed at ERIC elements. Lett Appl Microbiol 25:17–21
7. Harwood CS, Burchhardt G, Herrmann H, Fuchs G (1998) Anaerobic metabolism of aromatic compounds via the benzoyl-CoA pathway. FEMS Microbiol Rev 22:439–458
8. Jaspers E, Overmann J (2004) Ecological significance of microdiversity: identical 16S rRNA gene sequences can be found in bacteria with highly divergent genomes and ecophysiologies. Appl Environ Microbiol 70:4831–4839
9. Liu B, Zhang F, Feng X, Liu Y, Yan X, Zhang X et al (2006) *Thauera* and *Azoarcus* as functionally important genera in a denitrifying quinoline-removal bioreactor as revealed by microbial community structure comparison. FEMS Microbiol Ecol 55:274–286
10. Manefield M, Whiteley AS, Griffiths RI, Bailey MJ (2002) RNA stable isotope probing, a novel means of linking microbial community function to phylogeny. Appl Environ Microbiol 68:5367–5373
11. Mao Y, Zhang X, Zhang B, Zhao L (2008) Specific-PCR and denaturing gradient gel electrophoresis assisting isolation of *Thauera* spp. from a coking wastewater treatment plant. Acta Microbiologica Sinica (Wei Sheng Wu Xue Bao) 48:1634–1641
12. Mao Y, Zhang X, Yan X, Liu B, Zhao L (2008) Development of group-specific PCR-DGGE fingerprinting for monitoring structural changes of Thauera spp. in an industrial wastewater treatment plant responding to operational perturbations. J Microbiol Methods 75:231–236
13. Mechichi T, Stackebrandt E, Gad’on N, Fuchs G (2002) Phylogenetic and metabolic diversity of bacteria degrading aromatic compounds under denitrifying conditions, and description of *Thauera phenylacetica* sp. nov., *Thauera aminoaromatica* sp. nov., and *Azoarcus buckelii* sp. nov. Arch Microbiol 178:26–35
14. Probian C, Wulfing A, Harder J (2003) Anaerobic mineralization of quaternary carbon atoms: isolation of denitrifying bacteria on pivalic acid (2,2-dimethylpropionic acid). Appl Environ Microbiol 69:1866–1870
15. Rivera IG, Chowdhury MA, Huq A, Jacobs D, Martins MT, Colwell RR (1995) Enterobacterial repetitive intergenic consensus sequences and the PCR to generate fingerprints of genomic DNAs from *Vibrio cholerae* O1, O139, and non-O1 strains. Appl Environ Microbiol 61:2898–2904
16. Satoh H, Yamakawa T, Kindaichi T, Ito T, Okabe S (2006) Community structures and activities of nitrifying and denitrifying bacteria in industrial wastewater-treating biofilms. Biotechnol Bioeng 94:762–772
17. Scholten E, Lukow T, Auling G, Kroppenstedt RM, Rainey FA, Diekmann H (1999) *Thauera mechernichensis* sp. nov., an aerobic denitrifier from a leachate treatment plant. Int J Syst Bacteriol 49 Pt 3:1045–1051
18. Shinoda Y, Sakai Y, Uenishi H, Uchihashi Y, Hiraishi A, Yukawa H et al (2004) Aerobic and anaerobic toluene degradation by a newly isolated denitrifying bacterium, Thauera sp. strain DNT-1. Appl Environ Microbiol 70:1385–1392
19. Song B, Palleroni NJ, Haggblom MM (2000) Description of strain 3CB-1, a genomovar of *Thauera aromatica*, capable of degrading 3-chlorobenzoate coupled to nitrate reduction. Int J Syst Evol Microbiol 50 Pt 2:551–558
20. Song B, Palleroni NJ, Kerkhof LJ, Haggblom MM (2001) Characterization of halobenzoate-degrading, denitrifying *Azoarcus* and *Thauer*a isolates and description of *Thauera chlorobenzoica* sp. nov. Int J Syst Evol Microbiol 51:589–602
21. Thomsen TR, Kong Y, Nielsen PH (2007) Ecophysiology of abundant denitrifying bacteria in activated sludge. FEMS Microbiol Ecol 60:370–382
22. Valle A, Bailey MJ, Whiteley AS, Manefield M (2004) N-acyl-l-homoserine lactones (AHLs) affect microbial community composition and function in activated sludge. Environ Microbiol 6:424–433
23. Zhang M, Tay JH, Qian Y, Gu XS (1998) Coke plant wastewater treatment by fixed biofilm system for COD and NH3-N removal. Water Res 32:519–527
24. Zoetendal EG, Akkermans ADL, De Vos WM (1998) Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. Appl Environ Microbiol 64:3854–3859