World J Microbiol Biotechnol (2012) 28:447–452
DOI 10.1007/s11274-011-0834-z

ORIGINAL PAPER

Isolation and characterization of sulfonamide-degrading bacteria *Escherichia* sp. HS21 and *Acinetobacter* sp. HS51

Wei-Wei Zhang · Ying-Ying Wen · Zong-Liang Niu · Kun Yin · Dong-Xue Xu · Ling-Xin Chen

Received: 6 March 2011 / Accepted: 30 June 2011 / Published online: 9 July 2011

Abstract With the intensive application of sulfonamides in aquaculture and animal husbandry and the increase of sulfonamides discharged into the environments, there is an increasing need to find a way to remediate sulfonamide-contaminated environments. Two bacterial strains capable of degrading sulfonamides, HS21 and HS51, were isolated from marine environments. HS21 and HS51 were identified as members of *Escherichia* sp. and *Acinetobacter* sp., respectively, based on 16S rRNA gene sequencing. Degradation of each sulfonamide by *Escherichia* sp. HS21 and *Acinetobacter* sp. HS51 was characterized using capillary electrophoresis. About 66 or 72% of sulfapyridine and 45 or 67% of sulfathiazole contained in the media was degraded by *Escherichia* sp. HS21 or *Acinetobacter* sp. HS51, respectively, after incubation for 2 days. The supernatant from culture of *Escherichia* sp. HS21 or *Acinetobacter* sp. HS51 grown in sulfapyridine or sulfathiazole contained media had much attenuated cytotoxicity against HeLa cells. These results suggest that *Escherichia* sp. HS21 and *Acinetobacter* sp. HS51 are new bacterial resources for biodegrading sulfonamides and indicate the potential of isolated strains for the bioremediation of sulfonamide-polluted environments.

Keywords Sulfonamides · *Escherichia* sp. · *Acinetobacter* sp. · Degradation · Cytotoxicity

W.-W. Zhang · Y.-Y. Wen · Z.-L. Niu · K. Yin · L.-X. Chen
Key Laboratory of Coastal Environmental Processes, Chinese Academy of Sciences, Yantai, China

W.-W. Zhang · Y.-Y. Wen · Z.-L. Niu · K. Yin · L.-X. Chen
Shandong Provincial Key Laboratory of Coastal Zone Environmental Processes, 17 Chunhui Road, Yantai 264003, China

W.-W. Zhang · Y.-Y. Wen · Z.-L. Niu · K. Yin · L.-X. Chen (✉)
Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, 17 Chunhui Road, Yantai 264003, China
e-mail: lxchen@yic.ac.cn

Y.-Y. Wen · K. Yin
Graduate University of Chinese Academy of Sciences, Beijing 100049, China

D.-X. Xu
Department of Aquaculture, China Agriculture University (Yantai), 2006 Binhai Road, Yantai 264670, China

Introduction

Sulfonamides, derived from sulfanilamide (p-aminobenzenesulfonamide), are synthetic antimicrobials used as common pharmaceuticals in aquaculture, agriculture, and animal husbandry for preventing and treating infection diseases caused by bacteria or other microorganisms, including treating respiratory and urinary tract infections in humans (Holm et al. 1995; Ternes 1998; Hirsch et al. 1999; Jones et al. 2001; Sukul and Spiteller 2006). They work by providing a bacteriostatic effect, preventing the cells from growing by inhibiting the production of folic acid, which is required for bacterial growth (Craig and Stitzel 1994). Sulfonamides have also been recognized as an inhibitor of carbonic anhydrases, a family of zinc-containing enzymes implicated in a number of diseases such as arthritis and cancer, and thus, it is used to treat a range of disorders, including oedema, glaucoma, obesity, cancer, epilepsy and osteoporosis (Supuran 2008, 2011; Erdem et al. 2011). Only part of the sulfonamides ingested by humans or animals is absorbed and utilized by the body, and the rest are

excreted in faeces, urine or manure as metabolites or in the original form (Heberer 2002; Loffler and Ternes 2003).

Due to the improper and untreated disposal of sulfonamides in feed, sulfonamides and their metabolites are widespread in the environments and have frequently resulted in the contamination of water or soil (Halling et al. 1998; Fent et al. 2006; Ye et al. 2007; Barnes et al. 2008; Focazio et al. 2008). Sulfonamides are now widely recognized as environmental contaminants (Sukul and Spiteller 2006). One problem caused by the increasing concentration of sulfonamides in aquatic environments is that residual sulfonamides enhance the resistances of pathogenic bacteria to these substances and lead to the development of more antibiotic-resistant bacteria. Sulfonamides also act as micropollutants that disturb the normal structure and function of microflora (Halling et al. 1998; Witte 2000).

Some studies have focused on the removal of sulfonamides from the environments, but little research has been done on the biodegradation of sulfonamides (Jones et al. 2001; Heberer 2002; Sukul and Spiteller 2006; Kim et al. 2007). Biodegradation of sulfonamides present in wastewater treatment plants (WWTPs), surface water, manure and soils was summarized by Jesús García-Galán et al. (2008). Sulfonamides appeares to resist natural biodegradation rather strongly, which is reflected in the high frequency of their detection in streams and rivers (Kim et al. 2007; Vieno et al. 2007; Barnes et al. 2008; Focazio et al. 2008). Kuhn and Suflita (1989) reported the anaerobic biodegradation of para-toluenesulfonamide and benzenesulfonamide in aquifer slurries from a sulphate-reducing and methanogenic site. Richter et al. (2007, 2008a, b) determined that considerable removal of para-toluenesulfonamide (~90% reduction) during wastewater and drinking water treatment was performed by adapted microorganisms that were only present in polluted groundwater. Li and Zhang (2010) reported that the removal efficiencies of different sulfonamides after 48 h in saline sewage systems were 53.4 and 39.1% by microbial communities. It is clear that most of the studies dealt with degradation of sulfonamide mixtures by micropopulations and that studies on specific sulfonamide biodegradation by pure culture appear limited. Until now, no specific bacterial strain has been isolated, identified, characterized and used to degrade individual sulfonamide.

The main objective of this study was to isolate, identify and characterize sulfonamide-degrading bacterial strains from marine environments. Six sulfonamides, containing sulfadimidin, sulfadoxine, sulfamerazine, sulfapyridine, sulfathiazole, and sulfadiazine, were tested for biodegradation feasibility, and capillary electrophoresis (CE) was used to characterize sulfonamide-degrading activity. The cytotoxicity of the metabolites of sulfapyridine and sulfathiazole generated by bacterial degradation was also investigated to determine if there was any reduction in cytotoxicity.

Materials and methods

Chemicals and media

Analytical grade sulfadimidin, sulfadoxine, sulfamerazine, sulfapyridine, sulfathiazole, sulfadiazine and chromatographic grade acetonitrile were purchased from J&K Chemical Inc. (Beijing, China). The structures of the six sulfonamides are shown in Fig. 1. All other chemicals used were at the highest purity available commercially. The growth media consisted of (g l^{-1}) $Na_2HPO_4{\cdot}12H_2O$ 3, KH_2PO_4 1, NaCl 3, $MgSO_4$ 0.3, and tryptone 2.5, and the media was adjusted to pH 6.0–8.0. All the isolated strains were cultured at 28°C. If necessary, each sulfonamide was added into the media to a final concentration of 10 mg l^{-1}.

Determination of the concentration of sulfonamides using CE

Samples were analyzed for the concentration of each sulfonamide on a P/ACE MDQ CE system (Beckman Instruments, Fullerton, CA, USA) in conjunction with a diode-array detector (DAD) monitoring at 254 nm. Separation was performed at 25°C, using an applied voltage of 25 kV for 5 min. The buffer was prepared by freshly mixing 20 mM sodium dihydrogen phosphate and 10%

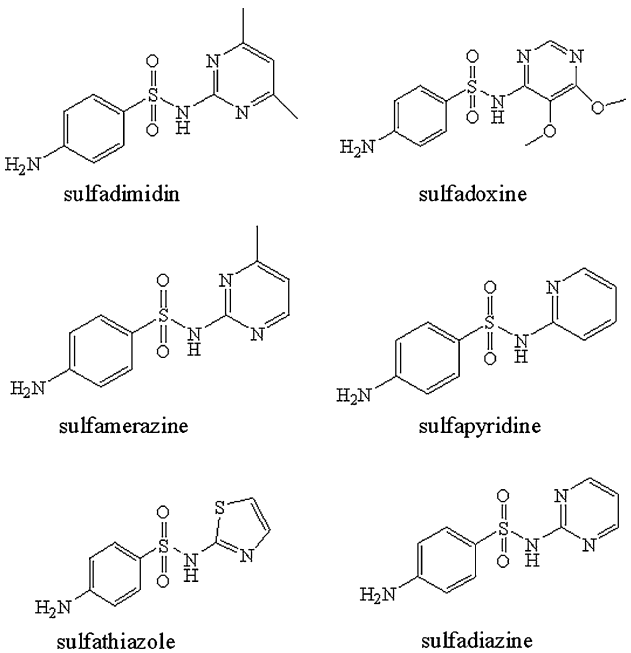

Fig. 1 Structures of the six sulfonamides

acetonitrile, and pH was adjusted to 8.5 with 1 M phosphoric acid. Bare fused-silica capillaries (Yongnian Photoconductive Fiber Factory, Hebei, China) with 75 μm i.d., 375 μm o.d., a total capillary length of 50.2 cm, and an effective length of 40 cm were used for sulfonamide separations. Quantification of samples was based on peak areas.

Isolation and identification of bacterial strains

Seawater was collected from the Yantai coastal zone, concentrated 10–20 times by filtration through a 0.45 μm pore filter membrane and plated onto media supplemented with 1.2% agar and 10 mg l^{-1} sulfathiazole. The appeared strains were examed for the ability to degrade sulfonamide. Genomic DNA of the isolates capable of degrading sulfonamide was extracted according to the method described by Syn and Swarup (2000). The 16S rRNA genes of HS21 and HS51 were amplified by PCR with primers 8F (5′-AG AGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTAC CTTGTTACGACTT-3′) according to the method described by Lane et al. (1985). Sequencing was carried out by the Genomics Institute (Beijing, China).

Degradation of sulfonamide by bacteria

The cells of HS21 or HS51 were collected by centrifugation at 10,000*g* for 5 min at room temperature after culturing at 28°C until an OD_{600} of 1.0 was reached. The cell pellets were washed twice with sterilized phosphate buffer (PBS) and adjusted to an OD_{600} of approximately 1.0. Then, cells were inoculated into media containing 10 mg l^{-1} of each sulfonamide to a final concentration of 10^7 CFU ml^{-1} and incubated at 28°C for 2 days. The same media, containing 10 mg l^{-1} of each sulfonamide without inoculation of any bacteria, was used as the control. Supernatants from culture of HS21 and HS51, and the control media were collected, filtered and stored at −20°C for CE analysis. To determine whether the reduced concentration of sulfonamide in the supernatant was due to the biodegradation, the sulfonamide that may be adsorbed and intracellular accumulated was detected. Bacteria were cultured in 10 ml sulfonamide contained media at 30°C for 2 days and cells were collected by centrifugation at 10,000*g* for 5 min. The cells were resuspend in 200 μl buffer B (100 mM NaH_2PO_4, 10 mM Tris–Cl, 8 M urea, adjust pH to 8.0 using NaOH) and lysed for 1 h by gently vortexing. Then cell lysate was diluted into 1.8 ml water and the mixture was sonicated using a sonicator equipped with a microtip with six 10 s bursts at 200–300 W and a 10 s cooling period between each burst. Supernatant was collected by centrifugation at 10,000*g* for 10 min. The supernatant from control media, culture and cell lysate was used for CE analysis.

Cytotoxic effects of sulfonamides and their metabolites

Cytotoxicity analysis was performed according to the method described by Zhang et al. (2009). In brief, HeLa cells were cultured to confluence in 96-well plates. Supernatants from HS21 and HS51 cultured in the presence of sulfapyridine or sulfathiazole was collected, respectively. Then, the supernatant was ultrafiltered with Amicon Ultra-4 centrifugal filter devices (Millipore) to remove any molecules larger than 10 kDa. The treated supernatant or PBS was added into the wells. After incubation at 37°C for 48 h, the viability of the cells was determined using the MTT Cell Proliferation Assay. The cytotoxicity of the supernatant from non-inoculated media with sulfapyridine or sulfathiazole was considered to be 100%. The cytotoxicity of supernatants from culture of HS21 and HS51, grown in the media with sulfapyridine or sulfathiazole, was expressed as the percentage of supernatant from non-inoculated media with sulfapyridine or sulfathiazole.

Nucleotide sequence accession numbers

The nucleotide sequences of the 16S rRNA genes of HS21 and HS51 have been deposited in the GenBank database under accession numbers HQ876771 and HQ876772, respectively.

Results and discussion

Detection of sulfonamides using CE

Sulfadimidin, sulfadoxine, sulfamerazine, sulfapyridine, sulfathiazole, or sulfadiazine that was added into the media were detected using CE. Good separation was achieved using a buffer system composed of 20 mmol l^{-1} phosphate and 10% acetonitrile at pH 8.5. The applied voltage for CE was 25 kV, and the detection wavelength was set at 254 nm. As shown in Fig. 2, the migration times of sulfadimidin, sulfadoxine, sulfamerazine, sulfapyridine, sulfathiazole and sulfadiazine were 3.975, 4.233, 4.417, 3.283, 4.708 and 4.533 min, respectively. The results showed that each of the tested sulfonamide could be separated within 5 min and that detection of sulfonamides by CE was a simple and reagent-saving method compared with the HPLC method (Sun et al. 2007).

Screening, isolation and identification of sulfonamide-degrading bacteria

Seawater collected from the Yantai coastal zone was concentrated and plated onto media supplemented with 1.2% agar and 10 mg l^{-1} sulfathiazole. After incubation at 28°C

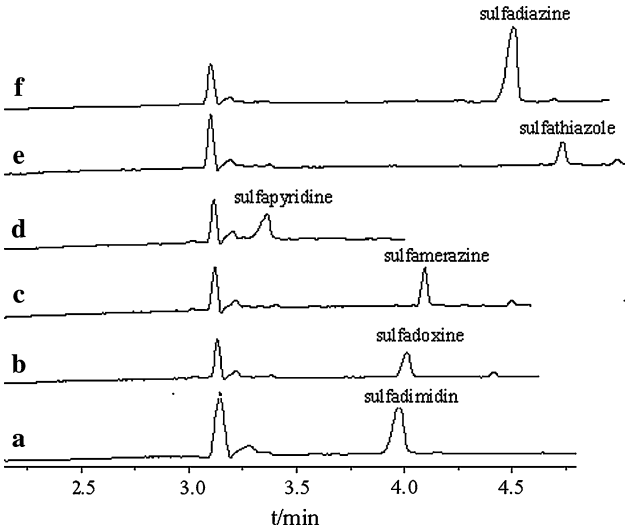

Fig. 2 Capillary electropherograms of each sulfonamide. The buffer was prepared by mixing 20 mM sodium dihydrogen phosphate and 10% acetonitrile and adjusting to pH 8.5 with 1 M phosphoric acid. Separation was performed at 25°C, using an applied voltage of 25 kV for 5 min. Sulfonamides were monitored using a DAD at 254 nm

for 2 days, colonies that had emerged were selected to test their sulfathiazole-degrading activity. Out of the 10 colonies that appeared, two strains had a clear ability to degrade sulfathiazole (Fig. 3b). These two strains were designated as HS21 and HS51. In order to genetically position the two isolates within the genus, the 16S rRNA genes of HS21 and HS51 were amplified by PCR, and the PCR products were purified and submitted directly for sequencing. Comparison of these sequences with the known 16S rRNA gene sequence data in the GenBank database indicated that 99% of the matches for the 16S rRNA gene sequence of HS21 were those of *Escherichia coli* strains KO11, w, CAIM 6 and FUA 1046, whose accession numbers were CP002516, CP002185, HM583969 and GQ222387, respectively; 100% of the matches for the 16S rRNA gene sequence of HS51 were those of *Acinetobacter calcoaceticus* strains CMST SSS5, LCR102 and *Acinetobacter* sp. Bap30, whose accession numbers were HM851460, FJ976611 and JF682491, respectively.

Degradation of sulfonamides by *Escherichia* sp. HS21 and *Acinetobacter* sp. HS51.

As shown in Fig. 3a and b, both the *Escherichia* sp. HS21 and *Acinetobacter* sp. HS51 strains showed obvious sulfapyridine-degrading and sulfathiazole-degrading activities from the comparison of the peak areas. After incubating *Escherichia* sp. HS21 or *Acinetobacter* sp. HS51 in the sulfonamide contained media for 2 days, 66 or 72% of the sulfapyridine was degraded by *Escherichia* sp. HS21 or *Acinetobacter* sp. HS51, respectively. Under the same conditions, 45 or 67% of the sulfathiazole contained in the media was degraded by *Escherichia* sp. HS21 or *Acinetobacter* sp. HS51, respectively. Sulfapyridine and sulfathiazole were undetectable in the cell lysate from *Escherichia* sp. HS21 or *Acinetobacter* sp. HS51 cultured in the media containing each sulfonamide. This result confirmed the fact that the reduced concentration of sulfonamide in the supernatant was due to the biodegradation by bacteria, ranther than the simple biosorption or accumulation. Moreover, *Acinetobacter* sp. HS51 demonstrated sulfadimidin-degrading, sulfadoxine-degrading and sulfadiazine-degrading activity under the tested conditions (Fig. 3c). These results showed that *Acinetobacter* sp. HS51 possessed better sulfonamide-degrading activity than *Escherichia* sp. strain HS21. As increasing research focuses on the biodegradation of antibiotics (Park and Choung 2007, 2010; Li and Zhang 2010), the biodegradation of sulfonamides by *Escherichia* sp. HS21 or *Acinetobacter* sp. HS51 may be exploited as an effective tool for degrading sulfonamides in the environment.

Cytotoxicity analysis of sulfonamides and their metabolites

Sulfapyridine and sulfathiazole that presented in the media significantly reduced the viability of HeLa cells. The OD_{470} of the wells treated with supernatant from non-inoculated sulfonamide contained media was about ten-fold lower than that of the wells treated with PBS. The supernatant from culture of *Escherichia* sp. HS21 or *Acinetobacter* sp. HS51 showed much attenuated cytotoxicity against HeLa cells, with 35 or 25% of cytotoxicity remained in the sulfapyridine contained media, and 53 or 32% of cytotoxicity remained in the sulfathiazole contained media, respectively (Fig. 4). It was postulated that the simultaneously decreased concentration of sulfapyridine or sulfathiazole in the media, or the presence of their less cytotoxic metabolites contributed to the lower cytotoxicity. Considering that there was still 34 or 28% of sulfapyridine and 55 or 33% of sulfathiazole remaining in the culture after degradation by *Escherichia* sp. HS21 or *Acinetobacter* sp. HS51, it was suggested that the metabolites of sulfapyridine and sulfathiazole generated by bacterial degradation showed negligible cytotoxicity to HeLa cells. This outcome is superior to the degradation products of tetracyclines, whose potency at the same concentration is equivalent to parental tetracycline, chlortetracycline, and oxytetracycline on both sludge and tetracycline-sensitive soil bacteria (Halling et al. 1998; Jeyasekaran et al. 2002).

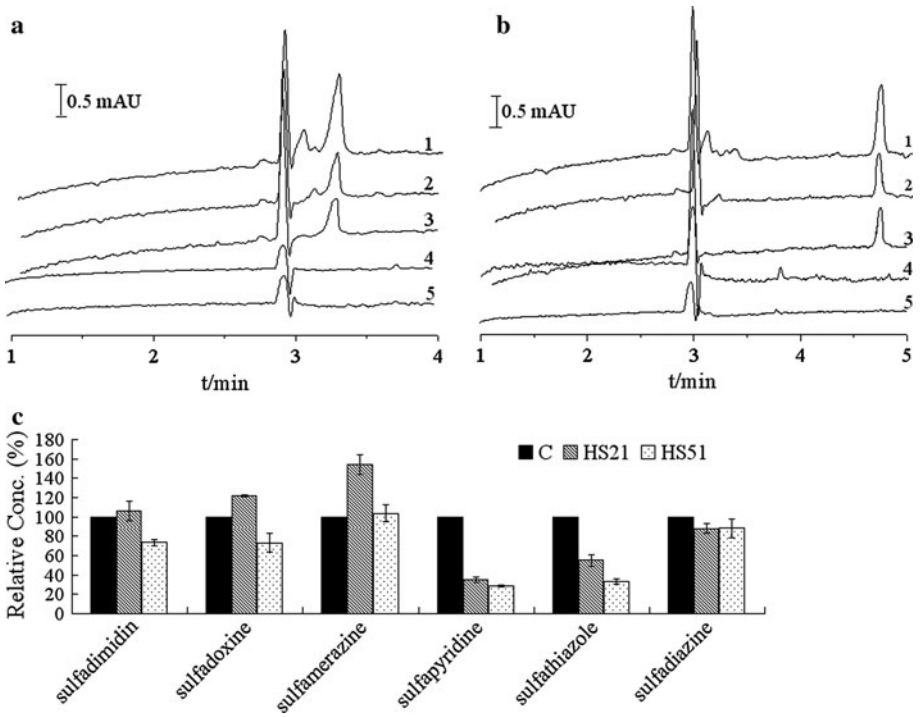

Fig. 3 Degradation of sulfonamides by *Escherichia* sp. HS21 and *Acinetobacter* sp. HS51. **a** CE analysis of sulfapyridine concentration in (*1*) supernatant from sulfapyridine contained media without inoculation of bacteria, (*2*) supernatant from culture of *Escherichia* sp. HS21 grown in the sulfapyridine contained media, (*3*) supernatant from culture of *Acinetobacter* sp. HS51 grown in the sulfapyridine contained media, (*4*) cell lysate of *Escherichia* sp. HS21 grown in the sulfapyridine contained media, and (*5*) cell lysate of *Acinetobacter* sp. HS51 grown in the sulfapyridine contained media. **b** CE analysis of sulfathiazole concentration in (*1*) the supernatant from sulfathiazole contained media without inoculation of bacteria, (*2*) supernatant from culture of *Escherichia* sp. HS21 grown in the sulfathiazole contained media, (*3*) supernatant from culture of *Acinetobacter* sp. HS51 grown in the sulfathiazole contained media, (*4*) cell lysate of *Escherichia* sp. HS21 grown in the sulfathiazole contained media, and (*5*) cell lysate of *Acinetobacter* sp. HS51 grown in the sulfathiazole contained media. **c** Relative concentration of each sulfonamide remaining in the supernatant after degraded by *Escherichia* sp. HS21 or *Acinetobacter* sp. HS51. Relative concentration was calculated based on the peak area. Data are the means for three independent experiments and are presented as the means ± SE

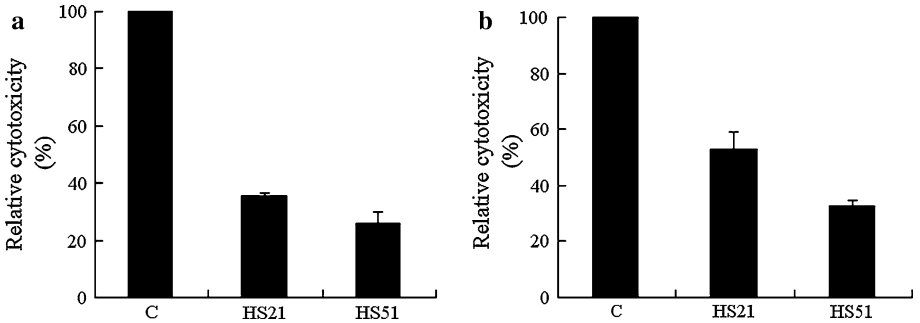

Fig. 4 Examination of the cytotoxic effects of different supernatants on cultured HeLa cells. Cell viability after the treatment was determined by the MTT method. **a** The relative cytotoxicity of supernatants from non-inoculated media containing 10 mg l^{-1} sulfapyridine, culture of *Escherichia* sp. HS21 and *Acinetobacter* sp. HS51 grown in the media containing 10 mg l^{-1} sulfapyridine, respectively. **b** The relative cytotoxicity of supernatants from non-inoculated media containing 10 mg l^{-1} sulfathiazole, culture of *Escherichia* sp. HS21 and *Acinetobacter* sp. HS51 cultured in the media containing 10 mg l^{-1} sulfathiazole, respectively. Data are the means for three independent assays and are presented as the means ± S.E

Conclusion

Two bacterial strains capable of degrading sulfonamides, especially sulfapyridine and sulfathiazole, were isolated and identified. The degradation of sulfonamides by *Escherichia* sp. HS21 and *Acinetobacter* sp. HS51 was characterized by CE, a simpler and more reagent-saving method for detecting sulfonamides than HPLC. The

metabolites of sulfapyridine and sulfathiazole generated by degradation of *Escherichia* sp. HS21 or *Acinetobacter* sp. HS51 showed negligible cytotoxicity to HeLa cells compared to the undigested sulfapyridine and sulfathiazole. This study supplies new sources for biodegrading sulfonamides, and the isolated strains might be used for the bioremediation of sites contaminated by sulfapyridine or sulfathiazole.

Acknowledgments This work was financially supported by the Innovation Projects of the Chinese Academy of Sciences grant KZCX2-EW-206, the National Natural Science Foundation of China (NSFC) grant 20975089, the Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences Kf201012, the Department of Science and Technology of Yantai City of China grant 2010235, and the 100 Talents Program of the Chinese Academy of Sciences.

References

Barnes KK, Kolpin DW, Furlong ET, Zaugg SD, Meyer MT, Barber LB (2008) A national reconnaissance of pharmaceuticals and other organic wastewater contaminants in the United States-I) groundwater. Sci Total Environ 402:192–200

Craig CR, Stitzel RE (1994) Modern pharmacology, 4th edn. Little Brown Co, New York

Erdem D, Yerlikaya E, Ceyhun SB, Demirdağ R, Şentürk M, Erdoğan O, Küfrevioğlu Öİ (2011) Inhibition effects of some sulfonamides on α-carbonic anhydrase from european seabass (*Dicentrachus labrax*). Hacettepe J Biol Chem 39:139–144

Fent K, Weston AA, Caminada D (2006) Ecotoxicology of human pharmaceuticals. Aquat Toxicol 76:122–159

Focazio MJ, Kolpin DW, Barnes KK, Furlong ET, Meyer MT, Zaugg SD, Barber LB, Thurman ME (2008) A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States-II) untreated drinking water sources. Sci Total Environ 402:201–216

Halling SB, Nors NS, Lanzky PF, Ingerslev F, Holten Lützhøft HC, Jørgensen SE (1998) Occurrence, fate and effects of pharmaceutical substances in the environment-a review. Chemosphere 36:357–393

Heberer T (2002) Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicol Lett 131:5–17

Hirsch R, Ternes T, Heberer K, Kratz KL (1999) Occurrence of the antibiotics in the aquatic environment. Sci Total Environ 225:109–118

Holm JV, Ruegge K, Bjerg PL, Christensen TH (1995) Occurrence and distribution of pharmaceutical organic compounds in the groundwater downgradient of a landfill (Grinsted, Denmark). Environ Sci Technol 29:1415–1420

Jesús García-Galán M, Silvia Díaz-Cruz M, Barceló D (2008) Identification and determination of metabolites and degradation products of sulfonamide antibiotics. TrAc–Trend Anal Chem 27:1008–1022

Jeyasekaran G, Karunasagar HallingSB, Sengeløv G, Tjørnelund J (2002) Toxicity of tetracyclines and tetracyline degradation products to environmentally relevant bacteria, including selected tetracycline-resistant bacteria. Arch Environ Contam Toxicol 42:263–371

Jones OAH, Voulvoulis N, Lester JN (2001) Human pharmaceuticals in the aquatic environment: a review. Environ Technol 22:1383–1394

Kim SD, Cho J, Kim IS, Vanderford BJ, Snyder SA (2007) Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters. Water Res 41:1013–1021

Kuhn EP, Suflita JM (1989) Anaerobic biodegradation of nitrogen-substituted and sulfonated benzene aquifer contaminants. Hazard Waste Hazard Mater 6:121–133

Lane D, Pace B, Olsen G, Stahl D, Sogin M, Pace N (1985) Rapid determination of 16S ribosomal sequences for phylogenetic analyses. Proc Natl Acad Sci 82:6955–6959

Li B, Zhang T (2010) Biodegradation and adsorption of antibiotics in the activated sludge process. Environ Sci Technol 44:3468–3473

Loffler D, Ternes TA (2003) Determination of acidic pharmaceuticals, antibiotics and ivermectin in river sediment using liquid chromatography-tandem mass spectrometry. J Chromatogr A 1021:133–144

Park HY, Choung YK (2007) Degradation of antibiotics (Tetracycline, Sulfathiazole, Ampicillin) using enzymes of glutathion S-transferase. Hum Ecol Risk Assess 13:1147–1155

Park HY, Choung YK (2010) Evaluation of the biodegradation feasibility of antibiotics by three bacteria involving glutathione S-transferases. Can J Civ Eng 37:814–819

Richter D, Dünnbier U, Massmann G, Pekdeger A (2007) Quantitative determination of three sulfonamides in environmental water samples using liquid chromatography coupled to electrospray tandem mass spectrometry. J Chromatogr A 1157:115–121

Richter D, Massmann G, Dünnbier U (2008a) Identification and significance of sulfonamides (p-TSA, o-TSA, BSA) in an urban water cycle (Berlin, Germany). Water Res 42:1369–1378

Richter D, Massmann G, Dünnbier U (2008b) Behaviour and biodegradation of sulfonamides (p-TSA, o-TSA, BSA) during drinking water treatment. Chemosphere 71:1574–1581

Sukul P, Spiteller M (2006) Sulfonamides in the environment as veterinary drugs. Rev Environ Contam Toxicol 187:67–101

Sun HW, Ai LF, Wang FC (2007) Quantitative analysis of sulfonamide residues in natural animal casings by HPLC. Chromatographia 66:333–337

Supuran CT (2008) Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nat Rev Drug Discov 7:168–181

Supuran CT (2011) Carbonic anhydrase inhibition with natural products: novel chemotypes and inhibition mechanisms. Mol Divers 15:305–316

Syn CK, Swarup S (2000) A scalable protocol for isolation of large-sized genomic DNA with in an hour from several bacteria. Anal Biochem 278:86–90

Ternes TA (1998) Occurrence of drugs in German sewage treatment plants and rivers. Water Res 32:3245–3260

Vieno NM, Härkki H, Tuhkanen T, Kronberg L (2007) Occurrence of pharmaceuticals in river water and their elimination in a pilot-scale drinking water treatment plant. Environ Sci Technol 41:5077–5084

Witte W (2000) Ecological impact of antibiotic use in animal on different complex microflora: environment. Int J Antimicrob Agents 14:321–325

Ye S, Zhang KW, Yao ZW, Ma DY (2007) Occurrence of sulfonamide pharmaceuticals in water column around Bohai sea. J Dalian Marit Univ 33:71–74

Zhang WW, Hu YH, Wang HL, Sun L (2009) Identification and characterization of a virulence-associated protease from a pathogenic *Pseudomonas fluorescens* strain. Vet Microbiol 139:183–188