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Sewage treatment plant serves as a hot-spot reservoir of integrons and gene cassettes

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Abstract

This study investigated the occurrence and abundance of class 1 integrons and related antibiotic resistance genes (ARGs) in a sewage treatment plant (STP) of China. Totally, 189 bacterial strains were isolated from influent, activated sludge and effluent, and 40 isolates contained the integons with a complete structure. The intI1-carrying isolates were found to harbor two types of gene cassettes: *dfr17-aadA5* and *aadA2*, conferring resistances to trimethoprim and streptomycin, which were further confirmed by antimicrobial susceptibility analysis. Many other gene cassettes were carried on integron, including *qnrVC1, catB-8-blaoxa-10-aadA1-aac(6'), aadB-aacA29b, aadA2, aac(6')-1b, aadA6 and aadA12*, which were detected using DNA cloning. Quantitative real time PCR showed that over 99% of the integrons was eliminated in activated sludge process, but average copy number of integrons in given bacterial cells was increased by 56% in treated sewage. Besides integrons, other mobile gene elements (MGEs) were present in the STP with high abundance. MGEs and the associated ARGs may be wide-spread in STPs, which constitute a potential hot spot for selection of antibiotic resistant bacteria and horizontal transfer of ARGs.

Key words

Class 1 integron, Antibiotic resistance gene, Gene cassette, Mobile gene element, Sewage treatment plant, Bacterial strains

Introduction

Antibiotic resistance, conferred by a series of antibiotic resistance genes (ARGs), is becoming one of the major global public health issues that deserve more concerns (Caballero-Flores *et al.*, 2012), since increased introduction of antimicrobial agents into the environment via medical therapy, agriculture and animal husbandry may accelerate the development of antibiotic resistance (Allen *et al.*, 2010). Previous studies revealed the prevalence of antibiotic-resistant bacteria in various aquatic environments, including river water (Kumar *et al.*, 2010), storm water (Nagachinta and Chen, 2008), drinking water (Shi *et al.*, 2012), groundwater (Mackie *et al.*, 2006), river sediments (Rosewarne *et al.*, 2010) and treated sewage (Okoh *et al.*, 2010). Recently, growing evidences have shown that ARGs, as emerging pollutants, are widely distributed in water environments (Zhang *et al.*, 2009b).

Sewage treatment plant (STP) is well known as an important reservoir of ARGs encoding for resistance to various antibiotics including sulfamethoxazole/ trimethoprim (da Silva *et al.*, 2007; Zhang *et al.*, 2009c), β-lactam (Pellegrini *et al.*, 2009), aminoglycoside (Tennstedt *et al.*, 2003) and tetracycline (Zhang *et al.*, 2009a). STPs receive the antibiotic-resistant bacteria with the inflow sewage water originating from hospitals, private households, industry, and agriculture, and play important roles in recombination, exchange, and spread of environmental ARGs (Okoh *et al.*, 2010). The characteristics of high microbial density and diversity in biofilms and activated sludge may facilitate horizontal transfer of ARGs in STPs (Schlüter *et al.*, 2007).

Mobile genetic elements (MGEs) including integrons, insertion sequences and plasmids could play an important role in transferring ARGs in environment (Zhang *et al.*, 2011). As one type of mobile genetic elements, integron plays a major role in multi-drug resistance and ARG horizontal transfer among bacterial species, due to its ability to capture gene cassettes from the environment and incorporate them by site-specific recombination (Lu *et al.*, 2010; Rosewarne *et al.*, 2010). So far, at least five classes of mobile integrons have been characterized, and class 1 integrons are the most common type detected in the environment (Mazel, 2006). The 5' conserved segment of class 1 integron harbors an integronase gene (*intI1*) encoding a site-specific integrase responsible for integration and excision of gene cassettes. Its 3' conserved segment at downstream of the gene cassette usually contains the gene *qacEΔ1* encoding a small exporter protein that confers resistance to quaternary ammonium compounds and the gene *sulI* encoding a dihydropteroate synthetase for sulphonamide resistance (Partridge *et al.*, 2009).

Currently, concerns mainly focus on characterization of integrons and antibiotic-resistance gene cassettes recovered from clinical or environmental bacterial species. However, little is known about elimination and fate of integrons and gene cassettes in STPs based on culture-independent surveys. This study aimed to investigate the occurrence and prevalence of integrons and the related ARGs in bacterial strains isolated from influent, activated sludge and effluent in one STP; and quantify the MGEs in the STP using quantitative real-time PCR (qRT-PCR) to assess the removal of MGEs in activated sludge process.

Materials and Methods

Sampling and DNA extraction: During March to August of 2010, sewage, activated sludge and treated wastewater were monthly sampled from the Jiangxinzhou Sewage Treatment Plant (Nanjing, China), with a daily average flow rate of 600,000 m^3. Conventional activated sludge process was used in the STP to treat domestic wastewater from a population about 1,500,000. Sewage samples were collected from influent of primary settler and the treated wastewater was sampled from effluent of secondary sedimentation tank. Activated sludge was sampled from aeration tank. The samples were kept on ice and transported to the laboratory for immediate processing within 3 h. The influent (100 ml) and the effluent (600 ml) were concentrated by filtration onto cellulose ester filters (0.45-μm-pore-size), and the sludge samples (550 μl) were centrifuged at 3,600 rpm for 10 min at 4 ^{o}C before the supernatant was discarded. The pellet was used for DNA extraction using the Genomic DNA Mini Preparation Kit (Beyotime Institute of Biotechnology, China). Extracted DNA was checked by electrophoresis on 1% agarose gel, and the concentration and purity were measured spectrophotometrically by determining absorbency at 260 nm and 280 nm.

Bacterial isolation and identification: Bacteria were isolated from the influent water, activated sludge and effluent water on MacConkey agar. The influent water and activated sludge samples were diluted by PBS buffer in a series of concentrations and 200 μl of the liquid was spread on MacConkey agar and cultured at 37^{o}C. Clones on MacConkey agar were randomly selected for restriction fragment length polymorphism (RFLP) analysis. In detail, the primer set of PCR (polymerase chain reaction) shown in Table 1 was used for amplification of a conserved sequence of 16S rDNA (1,502 bp). PCRs were performed in a mixed solution (30 μl) containing 1 × PCR buffer, 1.5 mM Mg^{2+}, 3 pmol of each primer, 100 μM dNTP, 100 ng of template DNA, and 1 U of Taq polymerase. PCR conditions were initial denaturation at 94 ^{o}C for 7 min, followed by 35 cycles of 94 ^{o}C for 1 min, 55 ^{o}C for 1 min, and 72 ^{o}C for 1.5 min, with a final extension at 72 ^{o}C for 10 min. The PCR products were purified using Cycle-Pure Kit (Omega, America) and digested with AluI and RsaI (TaKaRa, Japan). The strains were typed based on RFLP profile on 1% (w/v) agarose gel. Representative sequences of each RFLP type were randomly selected for DNA sequencing to determine the taxonomy affiliation. The DNA sequences obtained were submitted to GenBank for BLAST searches (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and species identification.

Specific PCRs of integron-related genes: Specific PCR was used to detect the genes *intI1*, *qacEΔ1* and *sulI* in the environmental samples and bacterial isolates using the primers listed in Table 1. PCR amplifications of these target genes were performed in a 30 μl reaction system containing 1 × PCR buffer, 1.5 mM Mg^{2+}, 3 pmol of each primer, 100 μM dNTP, 100 ng of template DNA, and 1 U of Taq polymerase (TaKaRa, Japan). PCR conditions were initial denaturation at 94 ^{o}C for 5 min, followed by 35 cycles of 94 ^{o}C for 30 sec, 55 ^{o}C for 30 sec, and 72 ^{o}C for 60 sec, with a final extension at 72 ^{o}C for 7 min. The strains simultaneously carrying the three genes were selected for PCR detection of gene cassettes using the primers 5'CS and 3'CS (Table 1). PCR conditions of gene cassettes were initial denaturation at 94 ^{o}C for 5 min, followed by 35 cycles of 94 ^{o}C for 45 s, 55 ^{o}C for 45 s, and 72 ^{o}C for 80 sec, with a final extension at 72 ^{o}C for 10 min. PCR products were analyzed by electrophoresis on 1% (w/v) agarose gel with ethidium bromide in 1× TAE buffer at 100 V for 20 min.

DNA cloning: PCR product of *intI1* and gene cassettes in activated sludge was purified using Cycle-Pure Kit (Omega, America) and cloned using pMD^{TM}19-T Vector (TaKaRa, Japan). PCR amplified inserts were cloned into plasmid vectors within *Escherichia coli*, which were selected by the white-blue plaque selection method. The cloned ARGs on gene cassettes were amplified by PCR using 3'CS and 5'CS primers to identify the type of ARGs by DNA

Table 1 : PCR and qRT-PCR primers of 16S rDNA, *intI1* (integronase gene), *qacEΔ1* (quaternary ammonium compounds resistance gene), *sulI* (sulphonamide resistance gene), *trbC* (conservative sequence of conjugative plasmid), *ISEcp1* (insertion sequence), and *tnpA/Tn21* (transposase gene).

Target gene	Primer	Sequence (5'-3')	References
intI1 (484 bp)	Forward	GGGTCAAGGATCTGGATTTCG	Mazel *et al.*, 2000
	Reverse	ACATGGGTGTAAATCATCGTC	
qacEΔ1 (230 bp)	Forward	ATCGCAATAGTTGGCGAAGT	Sandvang *et al.*, 1998
	Reverse	CAAGCTTTTGCCCATGAAGC	
sulI (433 bp)	Forward	CGGCGTGGGCTACCTGAACG	Kerrn *et al.*, 2002
	Reverse	GCCGATCGCGTGAAGTTCCG	
trbC (255 bp)	Forward	CGGYATWCCGSCSACRCTGCG	Zhang *et al.*, 2010
	Reverse	GCCACCTGYSBGCAGTCMCC	
ISEcp1 (299 bp)	Forward	CTTCATTGGCATTGATAAGTTAG	Zhang *et al.*, 2010
	Reverse	TGTAGGATCGGTTTCCCAGTTTC	
tnpA/Tn21 (317 bp)	Forward	ATGCCACGTCGTTCCATCCTGTCC	Dahlberg and Hermansson, 1995
	Reverse	CCGGGTCTGCTCCCGCTGGCC	
Gene cassette (variable)	Forward	GGCATCCAAGCAGCAAG	Lévesque *et al.*, 1995
	Reverse	AAGCAGACTTGACCTGA	
16S rDNA (1502bp)	Forward	AGAGTTTGATCATGGCTCAG	Acinas *et al.*, 2007
	Reverse	GGTTACCTTGTTACGACTT	

sequencing. The plasmids carrying target gene *intI1* were extracted and purified using AxyPrep Plasmid DNA Miniprep Kit (Axygen, America). The copy number of *intI1* per microliter plasmid DNA solution was calculated according to Liang *et al.* (2008). Calibration curve (*Ct* value versus Log value of initial target gene copy number per reaction) with six points was generated for qRT-PCR using tenfold serial dilution of the plasmid carrying target gene (Liang *et al.*, 2008; Zhang and Fang, 2006). Based on the calibration curves, *Ct* value of a test sample was used to calculate copy number of target gene, and then the latter was normalized against mass (nanogram) of the extracted DNA samples and the volume (milliliter) of sewage or sludge samples (Zhang *et al.*, 2009c).

qRT-PCR of MGEs: qRT-PCR was used to determine the abundance of *intI1* (class 1 integron), *ISEcp1* (insertion sequence), *tnpA/Tn21* (transposon), and *trbC* (conjugative plasmid) in influent water, activated sludge and effluent water sampled from the STP. The qRT-PCR primers are shown in Table 1. The final reaction volume was 20 µl, containing 10 µl SYBR Green Supper Mix (TaKaRa, Japan), plus 1 µl each primer (10 µM) and 8 µl template DNA or diluted plasmid carrying the target gene. Thermal cycling and fluorescence detection were conducted on a real-time rotary analyzer of Corbett Rotor-Gene™ 6000 using the software Rotor-Gene 6000 Series Software 1.7 (QIAGEN, the Netherlands). The qRT-PCR program consisted of an initial denaturation at 94 °C for 3 min, followed by 45 cycles of 94 °C for 30 sec, 55 °C for 30 sec, and 72 °C for 60 sec and a fluorescence acquisition step at 99 °C. All reactions were conducted in duplicate with ddH_2O as blank control. In order to correct for potential variations in extraction efficiencies, eubacterial 16S rRNA genes were quantified using the method recommended by López-Gutiérrez *et al.* (2004), so that MGEs abundance could be normalized to the total bacterial community.

Table 2 : The types of gene cassettes carried by the isolated strains and the related antibiotic susceptibility analysis results.

Strain	Species	Antibiotic susceptibility phenotype						Gene cassette type
		Ampi cillin	Pipera cillin	Tetra cycline	Sulfamet hoxazole	Trime thoprim	Strepto mycin	
AS-43[1]	*Klebsiella* sp.	R	R	R	R	S	R	*aadA2*
AS-49[1]	*Aeromonas salmonicida*	R	S	R	S	R	R	*aadA2*
AS-55[1]	*Escherichia coli*	R	R	S	S	R	R	*aadA2*
AS-58[1]	*Escherichia coli*	R	S	S	R	S	R	*aadA2*
AS-78[1]	*Escherichia coli*	S	S	S	S	S	R	*dfr17-aadA5*
E-13[2]	*Escherichia coli*	R	R	R	R	R	R	*dfr17-aadA5*
E-14[2]	*Escherichia coli*	M	R	R	R	R	R	*dfr17-aadA5 & aadA2*
E-49[2]	*Klebsiella* sp.	R	R	S	S	S	R	*aadA2*

Note: [1]Isolated from activated sludge; [2]Isolated from effluent water. R: resistant; S: sensitive; M: moderate.

Antibiotic susceptibility analysis: The strains containing gene cassettes were subject to antibiotic susceptibility analysis of ampicillin, piperacillin, tetracycline, sulfamethoxazole, trimethoprim and streptomycin (Table 2). The types of antibiotics were selected based on detection results of the ARGs by specific PCRs, The isolated strains were sent to the Shanghai Adicon Central Lab (Shanghai, China) for antibiotic susceptibility analysis using the disc diffusion method recommended by Clinical and Laboratory Standards Institute (2005).

GenBank accession number: The sequences of gene cassettes obtained in this study have been deposited in GenBank under accession No. HQ611040 to HQ611047 and JN588530 to JN588535.

Results and Discussion

Occurrence of class 1 intgrons in STPs: Specific PCRs showed that class 1 integronase gene *intI1* (on 5' conserved segment of class 1 integron), as well as *sulI* and *qacEΔ1* (on 3' conserved segment), occurred in each sample of the influent water, activated sludge and effluent water monthly collected from the STP of Nanjing City (Fig. 1). The PCR products of the three genes were checked by DNA sequencing. PCRs with the primer set of 5'CS and 3'CS showed that several types of gene cassettes with different sizes occurred in the samples of sewage and sludge (Fig. 1). Class 1 integrons were found to be widely distributed in the Jiangxinzhou STP of Nanjing, since *intI1*, *sulI* and *qacEΔ1* were detected in all the samples of influent, activated sludge and effluent during March to August of 2010. Previous studies have indicated that integrons carrying various ARGs frequently occurred in STPs (da Silva *et al.*, 2007; Zhang *et al.*, 2009a; 2009c). This study together with the previous reports shows that STPs serve as important reservoirs for integrons.

Integrons carried by bacterial strains: A total of 189 strains were isolated from influent water (54 stains), activated sludge (81 stains) and effluent water (54 stains) of the STP. Among the bacterial isolates, 57 ones were found to carry integronase gene *intI1*, including 11 ones from the influent, 25 ones from activated sludge and 21 ones from the effluent (Fig. 2). The detection frequency of *intI1*-carrying strain in the influent, activated sludge and effluent were 20.4%, 30.9% and 38.9%, respectively. Totally, 49 strains were found to carry *sulI* and 44 ones contained *qacEΔ1*. At the same time, PCRs showed that 40 strains simultaneously carried *intI1*, *sulI* and *qacEΔ*1, the required components for class 1 integrons with complete structure (Fig. 2). The proportion of isolated strains carrying class 1 integrons is comparable to other reports. Taviani *et al.* (2008) showed that 26% (five of 19) of antibiotic resistant *Vibrio* spp. isolated from surface urban water (river and sea) contained class 1 integrons, and

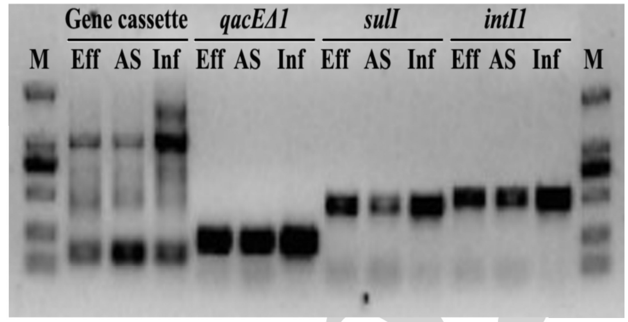

Fig. 1 : Electrophoresis image of RCR results for detection of the genes (*intI1*, *sulI*, *qacEΔ1* and gene cassettes) located on class 1 integrons present in the sewage treatment plant of Nanjing, China.

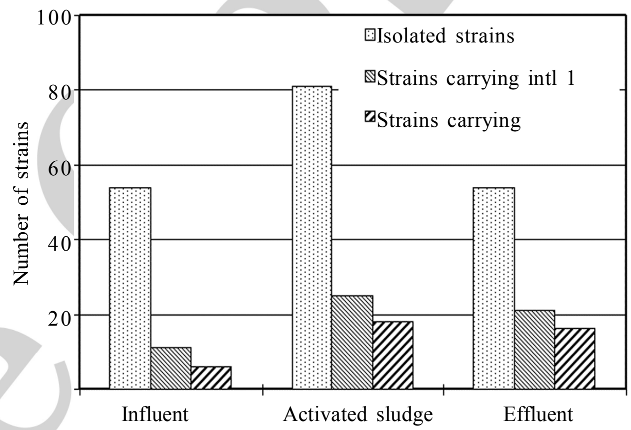

Fig. 2 : Number of integron-carrying isolates detected in influent water, activated sludge and effluent water of the sewage treatment

da Silva *et al.* (2007) found that 31 of 343 *Enterobacteria* strains isolated from raw and treated wastewater of a municipal treatment plant contained class 1 integrons. This study showed that 40 of 189 isolates carried class 1 integrons with complete structure including *intI1, sulI* and *qacEΔ1.* However, the detection frequency was found to be relatively higher in animal bodies (Zhang *et al.,* 2009e).

According to the types of ARGs in gene cassettes carried by the eight isolates, six antibiotics (including ampicillin, tetracycline, piperacillin, sulfamethoxazole, trimethoprim and streptomycin) were chosen for the antibiotic susceptibility analysis on the bacterial isolates. Table 2 shows that 7 bacterial strains were resistant to at least three types of the tested antibiotics, confirming that the strains carrying integrons were closely related to multiple resistances. Additionally, all the strains containing *aadA2* gene were resistant to streptomycin and the all the strains carrying *dfr17*, except for strain AS-78, were resistant to trimethoprim.

Characterization of gene cassettes on class 1 integrons of bacterial strains: PCRs were also performed to check the

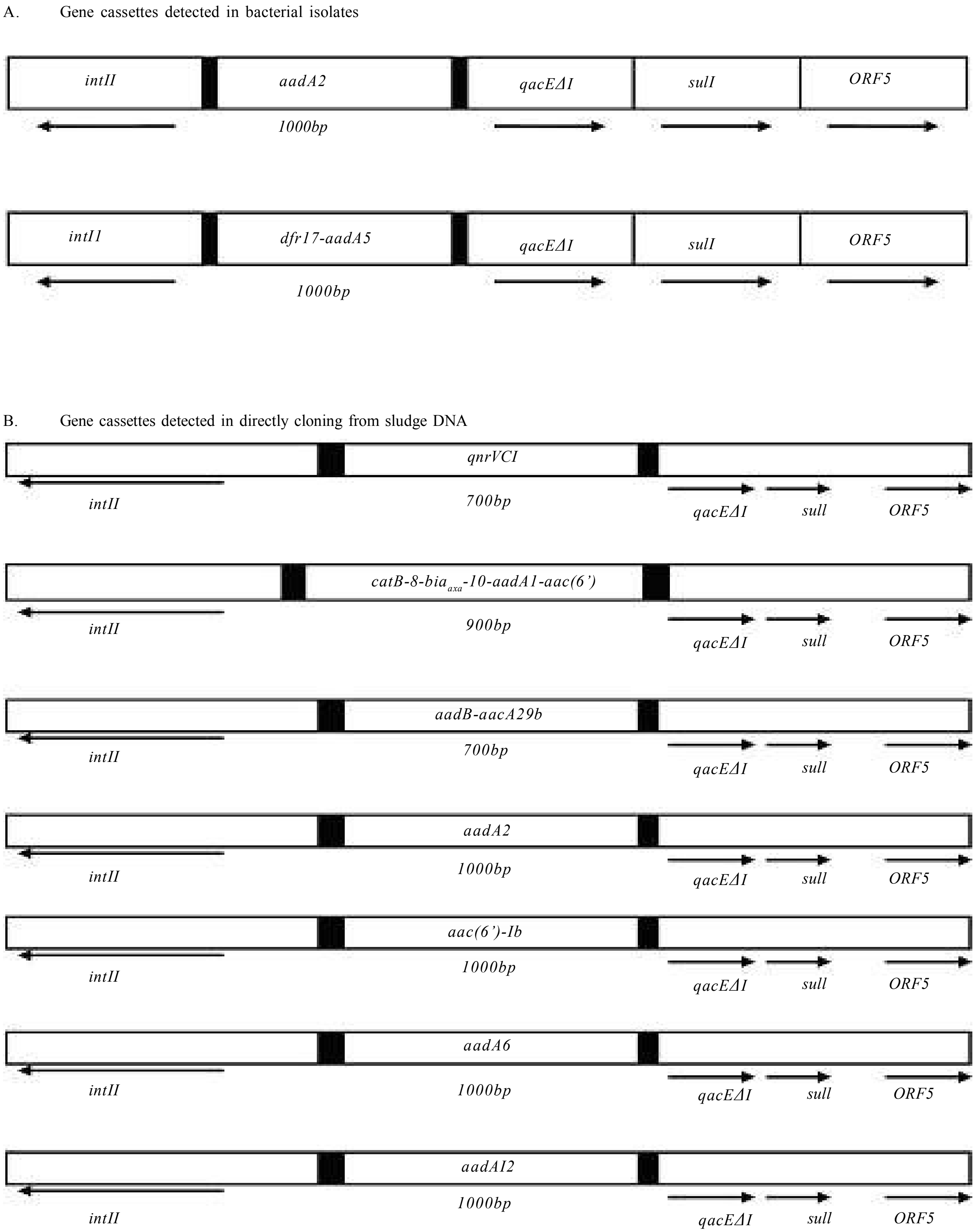

Fig. 3 : Gene cassettes located on class 1 integrons in bacterial isolates detected by specific PCRs (A) and in activated sludge detected by DNA cloning (B)

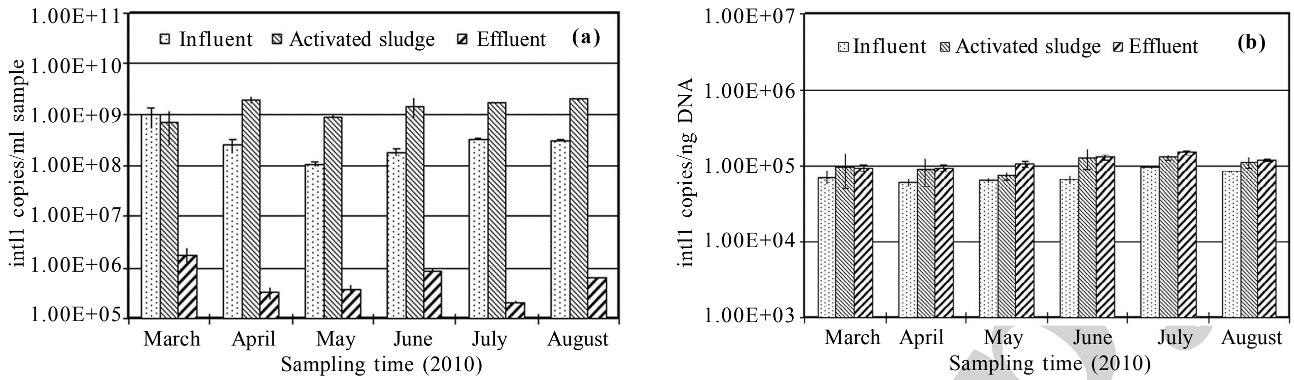

Fig. 4 : Abundance of *intI1* in influent water, activated sludge and effluent water of the sewage treatment plant normalized to volume of sewage or sludge samples (a) and mass of extracted DNA (b).

presence of gene cassettes in the 40 strains containing class 1 integrons with complete structure. In this study, eight strains were found to carry the gene cassette, which were grouped into two types. As shown in Fig. 3a, DNA sequencing and BLAST comparison revealed that the two types of gene cassette had high identities (> 99%) to the known gene cassettes *aadA2* (about 1,000 bp) and *dfr17-aadA5* (about 1,600 bp). 16S rDNA PCRs were also conducted for the phylogenetic analyses on the eight stains and BLAST searches showed that they were affiliated to *Escherichia coli*, *Klebsiella* sp. and *Aeromonas salmonicida* (Table 2). Recently, some studies have shown a frequent occurrence of gene cassettes *dfr17-aadA5* and *aadA2* carried by integrons in bacterial isolates from water environments (Kim *et al.,* 2008; Moura *et al.,* 2007) and animal or human bodies (Sáenz *et al.,* 2010; Xu *et al.,* 2009). However, limited information is available about the presence of the two types of gene cassettes in STPs. This study firstly revealed the occurrence of gene cassette *aadA2* in STPs, but a previous study (Zhang *et al.,* 2009c) showed that about 10% of the *Enterobacteria* strains isolated from activated sludge of a saline STP contained the gene cassette *dfr17- aadA5*.

Gene cassettes on class 1 integrons in activated sludge were also characterized by directly cloning PCR results of the cassettes in sludge DNA**.** Totally, 58 recombinants were found to carry different kinds of gene cassettes, including *qnrVC1, catB-8-bla*$_{oxa\text{-}10}$*-aadA1-aac(6'), aadB-aacA29b*, *aadA2, aac(6')-Ib, aadA6* and *aadA12* (Fig. 3b). This study suggested that the integrons in activated sludge carried amounts of different kinds of ARGs, and one integron could contain several ARGs and showed multi-resistance, demonstrating that STP environment is a huge gene pool of ARGs. Zheng *et al.* (2012) discovered *aadA1*, *aadA5* and *aacA4-aadA1* on class 1 integron and *sat2-aadA1* on class 2 integron. Moura *et al.* (2007) also revealed the presence of *aadA2* carried on integron in STP.

Elimination of integrons in the STP: Quantitative real-time PCR was used to investigate abundance and elimination of integrons in the STP. Fig. 4(a) shows the gene copies of class 1 integrons in the influent water, activated sludge and effluent water on a basis of volume (ml) of water or sludge samples. The abundance of *intI1* showed a temporally significant variation among the samples of influent, activated sludge and effluent samples. For each sampling time point, concentration of integrons in activated sludge or influent water was higher than that of effluent. The average removal rate of *intI1* was over 99% in the STP, indicating that activated sludge process had a high removal efficiency of integron.

In order to compare relative abundance in different samples, the copy number of *intI1* determined by qRT-PCR were normalized to mass (nanogram) of extracted DNA. As shown in Fig. 4(b), on a basis of DNA mass, the ratio of relative abundance of *intI1* in influent, activated sludge and effluent was 1.00:1.42:1.56, indicating that average copy number of class 1 integrons in one microbial cell was increased by about 56% after the sewage was treated using activated sludge process.

Activated sludge process reduced the abundance of integrons in one milliliter of sewage by decreasing the biomass, but it had no effect on the removal of the integrons in one microbial cell. Few previous studies investigated the effects of wastewater treatment processes and environmental factors on the removal of integrons. However, a recent study of Zhang *et al.* (2009b) demonstrated that activated sludge process had no effect on removal or degradation of the integrons. Sewage receives the bacteria previously exposed to antibiotics from private households and hospitals and is considered as a hotspot for integrons and various ARGs. Integrons go into STPs with sewage water, and most of them are located on the mobile plasmids plasmid or transposons in bacterial cells (Okoh *et al.,* 2010). Moreover, environmental conditions of activated sludge or

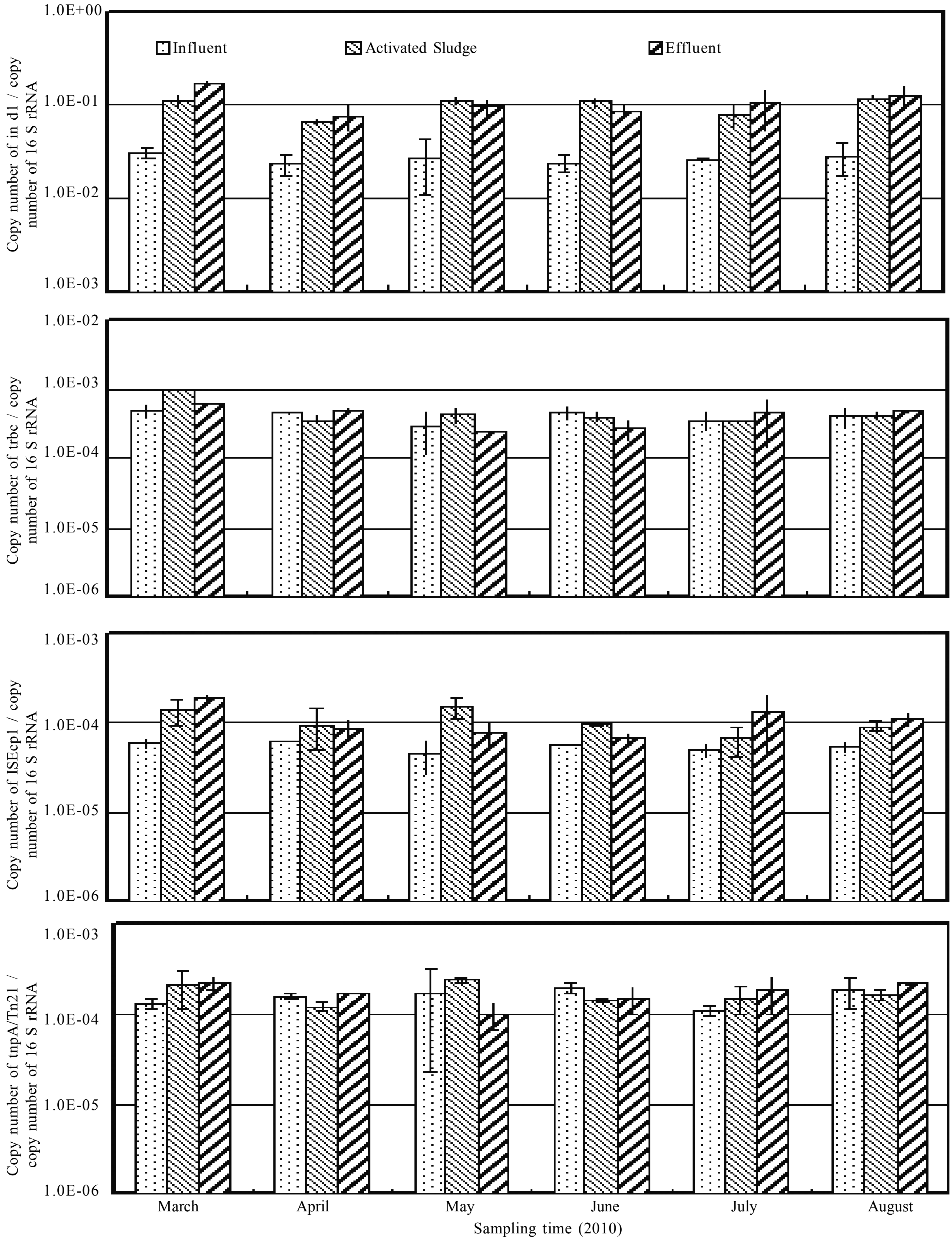

Fig. 5 : Abundance of mobile gene elements including *intI1* (class 1 integron), *ISEcp1* (insertion sequence), *tnpA/Tn21* (transposon), and *trbC* (conjugative plasmid) in the sewage treatment plant

biofilms facilitate horizontal transfer of the integrons from one host to another because of the nutritional richness and high bacterial density and diversity (Pellegrini *et al.,* 2009; Schlüter *et al.,* 2007). Therefore, microbial cells in the treated wastewater usually contain more copies of integrons than those in raw sewage.

Elimination of other MGEs in the STP: MGEs including *intI1*, *trbC*, *ISEcp*1 and *tnpA/Tn*21 were also quantified normalizing to 16S rRNA in influent, activated sludge and effluent (Fig. 5). All the four kinds of MGEs were detected in the STPs and integron had the highest abundance. Effluent contained more copies of *intI1* and *ISEcp*1 than influent, while the abundance of *trbC* and *tnpA/Tn*21 showed no evident increase after the process. This may result from the difference in molecular structure and bacterial location of the MGEs. The integronase *intI1* and insertion sequence *ISEcp*1 are often located on mobile plasmids in wastewater treatment plants, and the relative simple structure of integrons and insertion sequences may contribute to the horizontal transfer in the sludge and the higher abundance in the effluent (Tennstedt *et al.*, 2003; Zhang *et al.*, 2011). Similarly, Woodford *et al.* (2009) demonstrated high levels of integrons and other MGEs occurring in STPs. Some MGEs contain different kinds of ARGs, such as *ISE*cp1-$bla_{CTX-M-3}$, showing that MGEs may facilitate ARGs transfer, which brings great threats to human health.

SYBR Green qRT-PCR method was used to quantify *intI1*, *trbC*, *ISEcp*1 and *tnpA/Tn*21 to assess the removal efficiency of the activated sludge process in the STP. SYBR Green is considered as a reliable method of determining gene abundance in the environment and has been widely used to quantify integrons in STPs (Zhang *et al.,* 2009c), creek sediments (Hardwick *et al.,* 2008) and natural water environments (Zhang *et al.,* 2009d), as well as animal waste (Yu *et al.,* 2005). This study showed that most integrons were removed by the activated sludge process in the STP in terms of relative concentration based on sample volume. However, on a basis of 16S rRNA, the abundance of *intI1* and *ISEcp*1 was significantly increased after the treatment process, suggesting that reduction in microbial concentration played an important role in the removal of MGEs and ARGs.

In conclusion, class 1 integron and the related ARGs, as well as other MGEs were found to be prevalent in the STP of Nanjing City (China). After activated sludge process, the MGEs can be effectively removed by reducing the biomass in the treated wastewater. However, the average copy number of *intI1* and *ISEcp*1 per microbial cell was significantly increased, indicating that STPs constitute a potential hot spot for selection of antibiotic resistant bacteria and horizontal gene transfer of ARGs. The treated sewage discharged from STPs represents a potential health risk for dissemination of the undesirable genetic traits. Therefore, greater efforts have to be made to comprehensively investigate the occurrence and spread of MGEs and the associated ARGs in STPs, and more advanced wastewater treatment processes should be developed to effectively reduce the potential environmental health risk.

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