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Bacterial diversity in activated sludge from a consecutively aerated submerged membrane bioreactor treating domestic wastewater

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Abstract

The bacterial diversity of activated sludge from submerged membrane bioreactor (SMBR) was investigated. A 16S rDNA clone library was generated, and 150 clones were screened using restriction fragment length polymorphism (RFLP). Of the screened clones, almost full-length 16S rDNA sequences of 64 clones were sequenced. Phylogenetic tree was constructed with a database containing clone sequences from this study and bacterial rDNA sequences from NCBI for identification purposes. The 90.6% of the clones were affiliated with the two phyla *Bacteroidetes* (50%) and *Proteobacteria* (40%), and β-, γ-, and δ-*Proteobacteria* accounted for 7.8%, 28.1%, and 4.7%, respectively. Minor portions were affiliated with the *Actinobacteria* and *Firmicutes* (both 3.1%). Only 6 out of 64 16S rDNA sequences exhibited similarities of more than 97% to classified bacterial species, which indicated that a substantial fraction of the clone sequences were derived from unknown taxa. Rarefaction analysis of operational taxonomic units (OTUs) clusters demonstrated that 150 clones screened were still insufficient to describe the whole bacterial diversity. Measurement of water quality parameter demonstrated that performance of the SMBR maintained high level, and the SMBR system remained stable during this study.

Key words: bacterial diversity; restriction fragment length polymorphism (RFLP); submerged membrane bioreactor (SMBR); 16S rDNA clone library

Introduction

How to keep membrane bioreactor (MBR) systems remaining efficient is still a topic of argument because of the lack of sufficient information on the development of microbial community structure. Most researches focused on the microbial community of activated sludge from conventional activated sludge (CAS) system. However, it was demonstrated that some differences existed between CAS and MBR in terms of microbial community structure (Gao *et al*., 2004), and the microbial populations in MBR were capable of degrading a wider range of substrates than cultures from a CAS (Chiemchaisri *et al*., 1992; Suwa *et al*., 1992).

Determination of microbial community structure is important in controlling biological wastewater treatment systems because degradation of organic matter depends on metabolically active microorganisms. Information is still highly limited on microbial community structure and interactions associated with metabolic activities. The shortage of information is due, in part, to the lack of reliable techniques for analyzing microbial community structure and diversities in environmental samples. Recently, 16S rRNA/DNA-based molecular methods with high degrees of precision and specificity have been widely used for environmental microbial studies (Wagner *et al*., 1994; Bond *et al*., 1995; Liu *et al*., 2001). The full-length 16S rDNA sequence contains variable and conserved regions, which accurately reflect the phylogenetic position of the corresponding 16S rDNA sequences and the total degree of diversity (Lane *et al*., 1985).

The objectives of this study were to disclose the microbial diversity of an aerated submerged MBR treating synthetic domestic wastewater and to establish a 16S rDNA clone library to enable a more precise identification of the bacterial species.

1 Materials and methods

1.1 Submerged membrane bioreactor (SMBR) setup and operation

Synthetic wastewater was used to avoid big fluctuations in the feed concentration. The synthetic wastewater was composed of domestic wastewater and chemical compounds for simulating high strength domestic wastewater.

A pilot-scale SMBR (320 L in volume) was operated continuously (Fig.1). A hollow fiber membrane module

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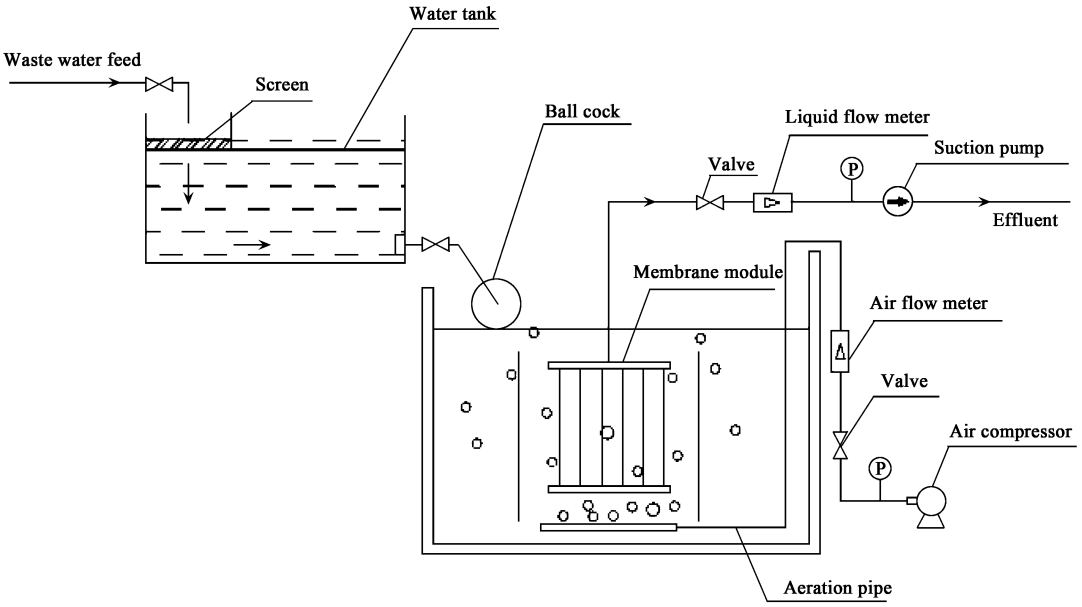

Fig. 1 Diagram of the pilot SMBR.

made from Polyvinylidene Fluoride (PVDF, nominal pore size 0.2 μm; inner diameter 0.6 mm) was submerged in the reactor. Oxygen was supplied through aeration pipes under the membrane module. Two baffles were inserted into the slots on the wall of the reactor to form the up flow area and down flow area to enhance the cross flow of mixed liquid (Liu *et al*., 2000). The effluent was withdrawn by a suction pump. The trans-membrane pressure was measured through the pressure gauge. $C_6H_{12}O_6{\cdot}H_2O$, $CO(NH_2)_2$, KH_2PO_4, $FeSO_4$, $MgSO_4{\cdot}7H_2O$, and $NaHCO_3$ were added in domestic wastewater to keep COD about 600 mg/L and COD:N:P = 125:10:1. $NaHCO_3$ and $MgSO_4{\cdot}7H_2O$ was added to maintain pH around 7 and supplement Mg^{2+} for meeting the need of microorganism growth, respectively. The hydraulic load was 42 L/h.

1.2 Sample preparation and measurement for water quality parameters

Samples of activated sludge were collected weekly during a month after the system had run stably for two weeks. DNAs were extracted from activated sludge samples using Soil DNA Kit (OMEGA, USA) following the manufacturer's instructions. Four DNA samples were pooled for PCR amplification of the 16S rDNA gene.

For evaluation of the stability and performance of SMBR system, chemical oxygen demand (COD), ammonia nitrogen (NH_4^+-N), total nitrogen (TN), and total phosphorus (TP) of influent and effluent from SMBR were measured every three days during this study.

1.3 16S rDNA library construction

PCR amplification of 16S rDNA genes was done by a primer set specific to bacteria: 8f (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492r (5'-TAC GGT TAC CTT GTT ACG ACT-3'). Amplification was carried out in a 25-μl reaction mixture containing 1.5 mmol/L $MgCl_2$, 0.2 mol/L each dNTP, 25 picomole each primer, 50 ng DNA template, and 2.5 U *Taq* DNA polymerase (Fermentas, USA) with reaction buffer supplied by the manufacturer. PCR was preheated at 94°C for 6 min prior to addition of *Taq* DNA polymerase; 30 cycles of 50 s at 94°C, 30 s at 50°C, and 90 s at 72°C, followed by a final extension of 12 min at 72°C. The PCR products were purified using a PCR purification kit (E.Z.N.A. Gel Extraction Kit, OMEGA, USA) following the manufacturer's instructions and then cloned into a PMD18-T vector (Takara) and transformed into *Escherichia coli* DH5α according to standard procedures (Sambrook *et al*., 1989).

1.4 RFLP screening of rDNA clones and sequencing

Nearly 150 clones were randomly selected and inoculated into 700 μl of LB broth supplemented with 100 μg ampicillin/ml. After incubating for 5 h at 37°C, 1 μl broth with *E. coli* was added to PCR reaction mixture as DNA template and PCR was conducted as above described but using primers RV-M and M13-47. Five-microliter aliquots of PCR products were ran on a 1% agarose gel to check for positive insert and fragment size. For the correct inserted clones, their residual PCR products were grouped according to the DNA patterns obtained by 3% agarose gel electrophoresis after *Hha I* and *Msp I* (Fermentas, USA) digestion (restriction fragment length polymorphism, RFLP). The RFLP patterns were compared visually and clones showing identical RFLP patterns were grouped into operational taxonomic units (OTUs). One representative clone from each group was selected for sequencing. Sequencing was performed on both strands using vector specific M13 forward and reverse primers by Invitrogen Biotechnology Co., Ltd., Shanghai, China.

1.5 Phylogenetic analysis

A sequence of 1,500 nucleotides from each clone was used for phylogenetic analysis. Sequences were compared with 16S rRNA gene sequences available

in the NCBI database (http://www.ncbi.nlm.nih.gov/) by BLASTn search. Multiple sequence alignments were performed using Clustal W version 1.8. Phylogenetic trees were constructed from evolutionary distances using the neighbor joining with Kimura 2 parameter distance in the PAUP 4.0 packages. A total of 1,000 bootstrap replicates were generated and a consensus tree was made. Representative sequences have been assigned GenBank accession numbers.

1.6 Rarefaction analysis and diversity indices

Clones with more than 97% sequence identity were considered as belonging to the same species (Buckley *et al*., 1998). Calculation of diversity indices and rarefaction analysis were applied to OTUs. Rarefaction calculations were done using the software Analytic Rarefaction (version 1.3, S. M. Holland, http://www.uga.edu/strata/software/). The program uses the rarefaction equations described by Hurlbert (1971) and Heck *et al*. (1975). Shannon diversity index (H) was calculated applying Eq.(1):

$$H = -\sum P_i \ln P_i \tag{1}$$

where, P_i is the number of clones in i OUT group divided by the total number of clones in clone library. Shannon evenness index (J) was calculated using Eq.(2):

$$J = H/\ln S \tag{2}$$

where, S is the total number of OTUs patterns. Coverage (C) was calculated using the Eq.(3):

$$C = 1 - n/N \tag{3}$$

where, n is the number of clones that occurred only once, and N is the total number of clones examined (Ravenschlag *et al*., 1999).

2 Results

Approximately 1,500 base pairs of bacterial 16S rDNA fragments were amplified. From the bacterial 16S rDNA clone library, 150 clones were randomly selected and each clone was subjected to PCR-RFLP. A total of 72 different banding patterns were detected. DNA isolated from a single representative clone from each RFLP group was PCR-amplified and sequenced on both strands. After excision of 8 chimeric sequences, a total of 64 different phylotypes were obtained (Table 1). The sequences obtained were subjected to phylogenetic analysis. All 64 OTUs were affiliated with sequences from six major lineages of the domain bacteria, namely, *Bacteroidetes*, β-, γ-, and δ-subdivisions of the *Proteobacteria*, *Firmicutes*, and *Actinobacteria* (Table 1, Figs.2 and 3).

2.1 *Bacteroidetes*

Phylogenetic analysis placed 32 OTUs (accounting for 50% of total OTUs) within *Bacteroidetes* (Fig.2), mainly grouped with *Flavobacteria* and *Sphingobacteria*. Phylogenetic analysis revealed that AS38 and AS56 (intersimilarity 0.99 and similarities with AM230484 both were 0.94) formed a common lineage with *Flavobacterium ferrugineum* (AM230484) and probably belonged to or were related closely to this taxon. Similarly, AS40 and AS43 (intersimilarity 0.98), and AS51 (similarities with AJ784892 and AB245369 were 0.94 and 0.96, respectively) might belong to *Haliscomenobacter hydrossis* (AJ784892) and *Dyadobacter ginsengisoli* (AB245369), respectively. AS22 and AS124 (intersimilarity 0.99) had high similarity (0.97) with uncultured lake bacterium S10.17 (AY752128), and low similarity (0.91) with *Niastella jeongjuensis* strain GR20-13 (DQ244076), which implied that both AS22 and AS124 probably belonged to one species and related to *Niastella jeongjuensis*. Five clones (AS15, AS26, AS55, AS71, and AS100) were related to uncultured bacterium clones and were difficult to be classified. The remnant OTUs in this phylum had their highest similarities with uncultured bacterium clones from different environmental samples.

2.2 *Proteobacteria*

Forty percent of the sequenced clones (26 out of 64) were included in the phylum *Proteobacteria*, clustering within three subdivisions of β-, γ-, and δ-*Proteobacteria*. Phylogenetic analysis revealed that β-Proteobacterial sequences AS6 formed a lineage with *Leptothrix ginsengisoli* (similarity 0.97) and hence probably belonged to this taxon. AS85 and AB252910 (similarity 0.99) formed a lineage with *Methylibium aquaticum* IMCC1728 (both similarities 0.99), therefore, they probably belonged to one species (Fig.3). Five γ-Proteobacterial sequences (AS7, AS20, AS24, AS76, and AS99) have the same nearest phylogenetic neighbor *Thiothrix eikelboomii* (AB042542, NCBI), and similarities were 0.99, 0.97, 0.92, 0.99, and 0.99, respectively, which revealed that except for AS24 they might belong to the same taxon. As to AS24, it must be closely related to this taxon. Clones AS1 and AS138 (interclone similarity 0.98) were the closest to AY332406 and were distinctly related to species from the genus *Photobacterium*. Similarly, clone AS87 was related to Bacteria H4 and *Serratia grimesii* (similarity 0.92 and 0.88). The residual clones clustered with various sequences from uncultured bacteria retrieved from activated sludge, lake water, soil, contaminated environments, and so on. Clones belonging to the subclass of δ-*Proteobacteria* formed three groups. One group consisting of clone AS105 (similarity 0.94) formed a common lineage with *Nannocystis exedens*, and the other two groups including clones AS48 and AS72 clustered with uncultured bacteria AY532573 and DQ139447, retrieved from uranium-contaminated aquifer and Roman catacombs, respectively.

2.3 Other bacterial divisions

Two clones (AS33 and AS42) were grouped within the phylum *Firmicutes* (low-GC and Gram-positive). Clone AS42 was related to EF694028 and EU015102 (both similarities were 0.99, and from activated sludge foam and membrane bioreactor suspension and biofilm,

Table 1 Summary of bacterial 16S rDNA sequenced clones

Clone No.	Accession No. of the nearest neighbor	16S rDNA similarity (%)	The nearest phylogenetic neighbor	Phylogenetic group
AS1	AY332406	100	*Photobacterium* sp. UCR13	γ-*Proteobacteria*
AS3	DQ640737	98	Uncultured *Bacteroidetes* bacterium clone Skagen115	*Bacteroidetes*
AS6	AB271046	97	*Leptothrix ginsengisoli*	β-*Proteobacteria*
AS7	AB042542	99	*Thiothrix eikelboomii* strain: COM-A	γ-*Proteobacteria*
AS8	DQ128336	92	Uncultured soil bacterium clone HSB CT52-D04	γ-*Proteobacteria*
AS10	DQ640737	97	Uncultured *Bacteroidetes* bacterium clone Skagen115	*Bacteroidetes*
AS12	EF572731	98	Uncultured bacterium clone S23-830	β-*Proteobacteria*
AS13	AF234717	99	Uncultured sludge bacterium S7	γ-*Proteobacteria*
AS15	AF513098	97	Uncultured bacterium clone 6	*Bacteroidetes*
AS20	AB042542	97	*Thiothrix eikelboomii* strain: COM-A	γ-*Proteobacteria*
AS21	AY662016	96	Uncultured bacterium clone 300E2-D11	γ-*Proteobacteria*
AS22	AY752128	97	Uncultured lake bacterium S10.17	*Bacteroidetes*
AS24	AB042542	92	*Thiothrix eikelboomii* strain: COM-A	γ-*Proteobacteria*
AS26	AF502209	96	Uncultured bacterium clone HP1A28	*Bacteroidetes*
AS28	EF061974	89	Uncultured *Bacteroidetes* bacterium clone XME60	*Bacteroidetes*
AS30	AF513095	98	Uncultured bacterium clone 47	*Bacteroidetes*
AS31	EF574438	90	Uncultured bacterium clone S25-782	Ungrouped
AS33	EF511169	87	Uncultured bacterium clone P5D1-416	*Actinobacteria*
AS34	AF513095	94	Uncultured bacterium clone 47	*Bacteroidetes*
AS35	EF590038	98	Uncultured bacterium clone D09	*Bacteroidetes*
AS38	AB286451	98	Uncultured bacterium gene clone: 0733	*Bacteroidetes*
AS40	AJ784892	94	*Haliscomenobacter hydrossis* strain DSM 1100	*Bacteroidetes*
AS42	EU015102	99	Uncultured bacterium clone M1-8	*Actinobacteria*
AS43	AF314423	98	Uncultured bacterium PHOS-HE45	*Bacteroidetes*
AS44	DQ628945	88	*Flavobacterium* sp. SOC A4(51)	*Bacteroidetes*
AS48	AY532573	87	Uncultured bacterium clone 1013-28-CG28	δ-*Proteobacteria*
AS50	EF648017	85	Uncultured α-*Proteobacterium* clone HB3	γ-*Proteobacteria*
AS51	AB255105	99	Uncultured bacterium gene clone: SC-111	*Bacteroidetes*
AS52	AF314423	99	Uncultured bacterium PHOS-HE45	*Bacteroidetes*
AS54	DQ640737	93	Uncultured *Bacteroidetes* bacterium clone Skagen115	*Bacteroidetes*
AS55	AY211071	93	Uncultured *Bacteroidetes* bacterium clone VC5	*Bacteroidetes*
AS56	DQ202140	99	Uncultured bacterium clone CJRA14	*Bacteroidetes*
AS59	AF502207	97	Uncultured bacterium clone HP1A82	*Bacteroidetes*
AS61	AF314423	89	Uncultured bacterium PHOS-HE45	*Bacteroidetes*
AS62	DQ640737	98	Uncultured *Bacteroidetes* bacterium clone Skagen115	*Bacteroidetes*
AS63	AF236010	91	β-*Proteobacterium* A0640	β-*Proteobacterium*
AS64	DQ640737	98	Uncultured *Bacteroidetes* bacterium clone Skagen115	*Bacteroidetes*
AS69	AF502208	88	Uncultured bacterium clone HP1A94	*Bacteroidetes*
AS71	EF516340	93	Uncultured bacterium clone FCPP437	*Bacteroidetes*
AS72	DQ139447	95	Uncultured *Acidobacteria* bacterium clone CAL6	δ-*Proteobacteria*
AS73	DQ443903	99	Uncultured bacterium clone LM12-4	*Actinobacteria*
AS74	AF314423	99	Uncultured bacterium PHOS-HE45	*Bacteroidetes*
AS75	EF632949	97	Uncultured bacterium clone Pia-s-90	γ-*Proteobacteria*
AS76	AB042542	99	*Thiothrix eikelboomii* strain: COM-A	γ-*Proteobacteria*
AS82	DQ640682	98	Uncultured *Bacteroidetes* bacterium clone Skagenf19	*Bacteroidetes*
AS84	AF314423	99	Uncultured bacterium PHOS-HE45	*Bacteroidetes*
AS85	AB252910	99	Uncultured β-*Proteobacterium*, clone: 015	β-*Proteobacterium*
AS86	AB252904	98	Uncultured β-*Proteobacterium*, clone: 233	β-*Proteobacterium*
AS87	AY345546	92	Bacterium H4	γ-*Proteobacteria*
AS98	AF234739	95	Uncultured sludge bacterium S43	γ-*Proteobacteria*
AS99	AB042542	99	*Thiothrix eikelboomii* strain: COM-A	γ-*Proteobacteria*
AS100	AF513098	97	Uncultured bacterium clone 6	*Bacteroidetes*
AS105	AJ233947	94	*Nannocystis exedens* strain Na e571	δ-*Proteobacteria*
AS110	AB042819	90	*Thiothrix eikelboomii*	γ-*Proteobacteria*
AS115	AY570603	88	Uncultured bacterium clone PL-1B5	*Actinobacteria*
AS124	AY752128	97	Uncultured lake bacterium S10.17	*Bacteroidetes*
AS128	EF574438	90	Uncultured bacterium clone S25-782	Ungrouped
AS131	AB200303	88	Uncultured bacterium clone: UTFS-OF09-d22-22	γ-*Proteobacteria*
AS134	AF314423	99	Uncultured bacterium PHOS-HE45	*Bacteroidetes*
AS136	DQ295906	96	Uncultured bacterium clone Cr-cl15	*Bacteroidetes*
AS137	AB200304	96	Uncultured bacterium clone: UTFS-OF09-d22-29	γ-*Proteobacteria*
AS138	AY302119	93	Uncultured bacterium clone DSBR-B020	γ-*Proteobacteria*
AS139	AF234749	94	Uncultured sludge bacterium H28	γ-*Proteobacteria*
AS140	EF020003	89	Uncultured *Bacteroidetes* bacterium, clone Elev-16S-1393	*Bacteroidetes*

respectively), therefore, it probably belonged to *Lactococcus* sp. CAU 28. Similarly, clone AS33 was closest to EF511169 and AY699289 (similarities were 0.87 and 0.86, respectively) and was related to species from the genus *Lactococcus*. Another two clones (AS73 and AS115) affiliated with the phylum *Actinobacteria*. Clone AS73's

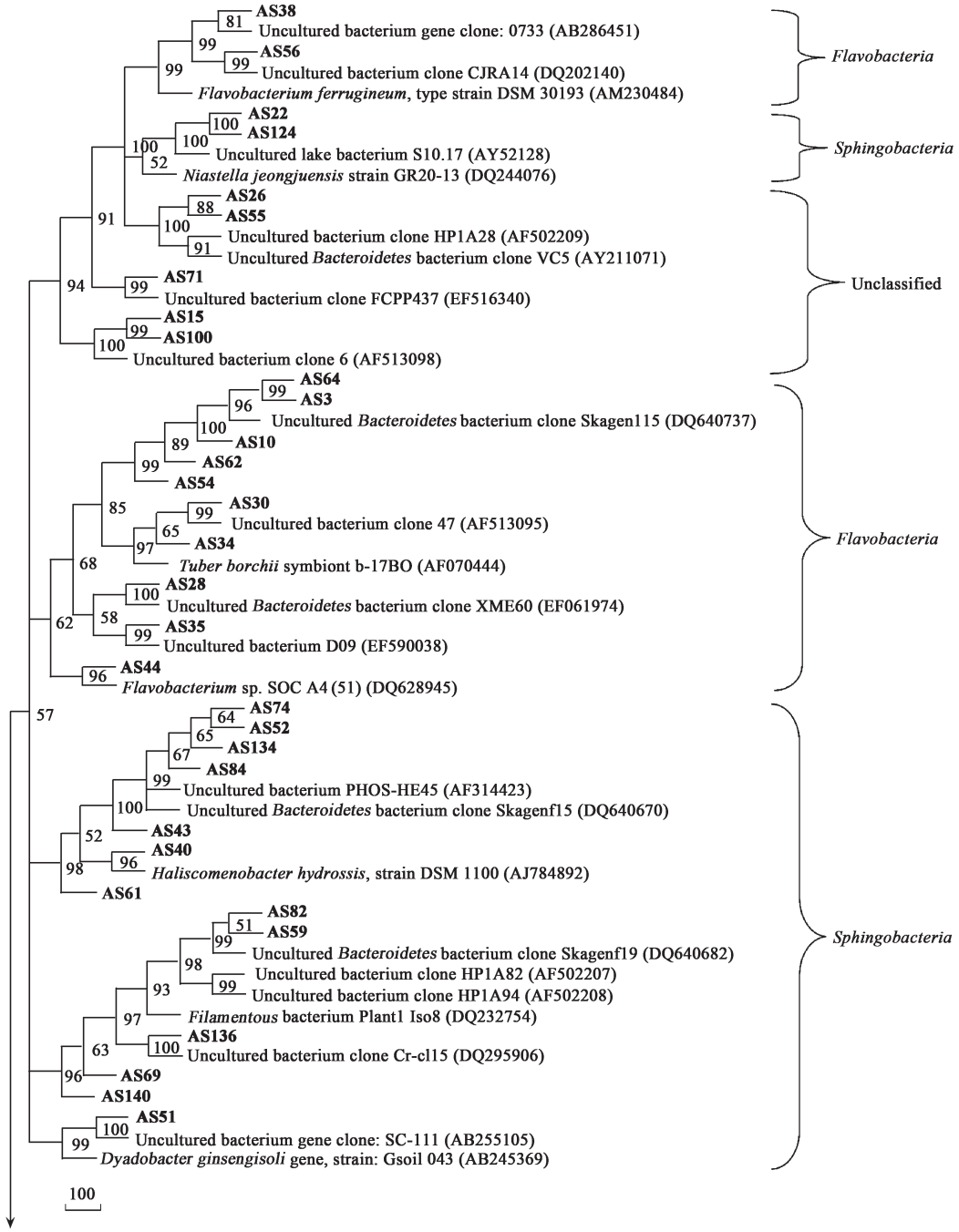

Fig. 2 Phylogenetic tree showing the relationship among bacterial 16S rDNA gene sequences belong to member of *Bacteroidetes* from activated sludge with reference sequences obtained through BLASTn analysis. Numbers in bold are sequences obtained from the present study. Bootstrap values (1,000 replicates) are shown at the nodes.

nearest phylogenetic neighbor was an uncultured bacterium clone LM12-4 (DQ443903, similarity 0.99) from sulfate-reducing bioreactor. Clone AS115 was close to uncultured bacterium clone PL-1B5 and *Nostocoida limicola* (similarities 0.88 and 0.87) from low-temperature biodegraded Canadian oil reservoir and reactor treating industrial waste, respectively. From the phylogenetic tree (Fig.3), clones AS31 and AS128 formed isolated groups and were difficult to be classified for their low similarities (both 0.90) with the nearest neighbor (EF574438).

2.4 Rarefaction analysis and diversity indices

Rarefaction analysis was done to estimate the diversity of the studied sample by screening 150 clones. The rarefaction curve (Fig.4) clearly showed no plateau and gave an indication of the extensive species richness. Shannon diversity index (H) was calculated to be 4.02, and Shannon evenness index (J) and Coverage (C) were found to be 0.97 and 82.9%, respectively.

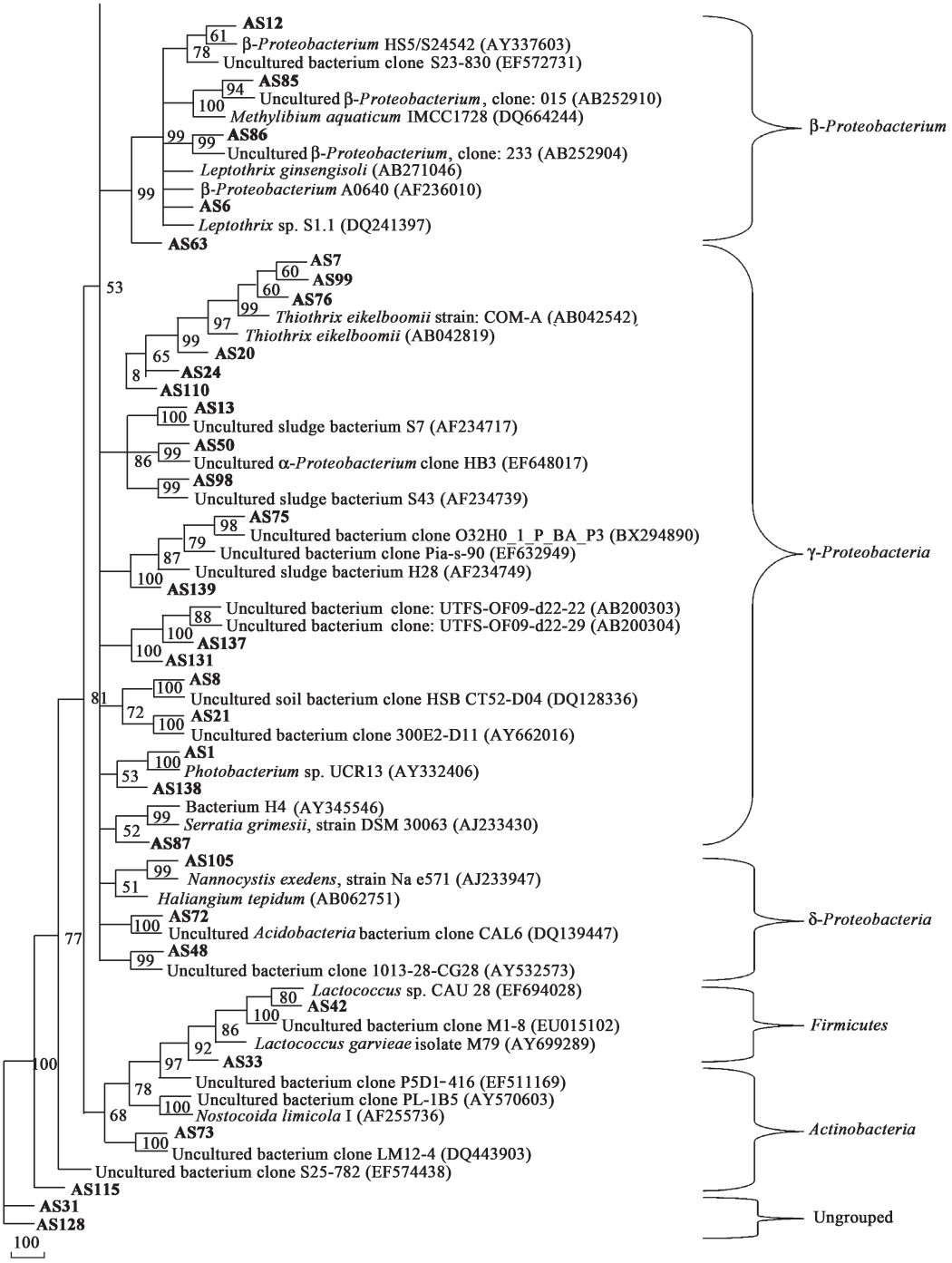

Fig. 3 Phylogenetic tree showing the relationship among bacterial 16S rDNA gene sequences belong to *Proteobacteria*, *Firmicutes*, and *Actinobacteria* from activated sludge with reference sequences obtained through BLASTn analysis. Numbers in bold are sequences obtained from the present study. Bootstrap values (1,000 replicates) are shown at the nodes.

2.5 Performance of the SMBR

Water quality parameters of influent, effluent, and removal rate in the MBR were measured (Table 2). The mean removal rates were all higher than 80%, and standard errors of these parameters were quite low.

Table 2 Mean treatment results for various parameters of water quality by SMBR (n = 10)

Parameter	Concentration*		Mean removal rate (%)
	Influent	Effluent	
COD (mg/L)	596.8 ± 7.76	14.4 ± 1.07	97.59
NH_4^+-N (mg/L)	25.12 ± 1.00	2.50 ± 0.11	90.06
TN (mg/L)	40.16 ± 1.14	6.21 ± 0.09	84.53
TP (mg/L)	3.97 ± 0.038	0.70 ± 0.03	82.33

* Data expressed as mean ± standard deviation.

3 Discussion

Prokaryotic diversity analysis based on 16S rDNA techniques could have a contribution to understand eco-

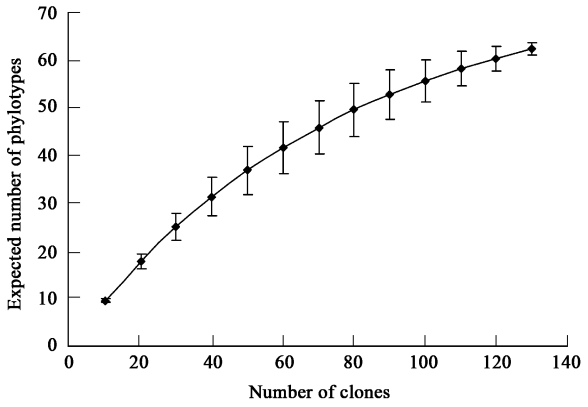

Fig. 4 Rarefaction curve for Bacteria from SMBR.

logically complex microbial community structures through the identification of populations. Without the limitations of culture techniques, the sequenced-based phylogenetic techniques provided a more complete picture of the community composition (Amann *et al*., 1995).

All clones from the clone library only focused on four known phyla and mostly affiliated with *Cytophaga-Flavobacterium-Bacteroidetes* (CFB) and *Proteobacteria* of the β-, γ-subdivisions. In the present result, it was interesting that appearing too large numbers of CFB and absence of α-*Proteobacteria* being general species in activated sludge samples were found. Possible reasons were that some strains could thrive and become the dominant communities under given nutrient and physiochemical conditions and number of selected clones was not big enough to include all strains, especially exiguous groups. Most (90%) of the 16S rDNA clones represented unidentified nonculturable bacteria, and only 6 out of 64 16S rDNA sequences exhibited similarities of more than 97% with classified bacterial species, which indicated that a vast fraction of the clone sequences were derived from unknown taxa. However, many clones were sludge-related, for example, AS7, AS20, AS24, AS76, and AS99 were related to *Thiothrix eikelboomii* (AB042542) belonging to filamentous bacterium (one of general species in activated sludge).

Numerical analysis of biodiversity provided interesting insights, and Shannon diversity index (*H*), Shannon evenness index (*J*) and Coverage (*C*) were found to be 4.02, 0.97, and 82.9%, respectively. The values of *H* and *J* showed that diversity and evenness of distribution of OTUs over the patterns were quite high and coverage of 82.9% demonstrated that a majority of microbial community was covered in this 16S rDNA clone library. The values obtained in the present study were quite high in comparison with estimates reported for sediments from the Lonar Soda Lake (Wani *et al*., 2006) and Victoria Harbor sediments in Hong Kong (Zhang *et al*., 2008), and low in comparison with a waste gas-degrading biofilter (Friedrich *et al*., 2002).

High diversity found in the present study implied that the methodology worked effectively for various phylogenetic groups. However, there were only a few clones related to the *Actinobacteria*, which were found to be present in considerable numbers in other systems, especially in foaming (Klein *et al*., 2007). Similar results were obtained for soil samples in which sequences affiliated with *Actinobacteria* accounted for only a minor fraction, as opposed to what could be expected from cultivation (Borneman *et al*., 1996). This discrepancy could be a consequence of poor lysis efficiency of cell wall or of selective PCR. As to how many kinds of endonuclease used were suitable, many authors have chosen two to four kinds of endonuclease commonly (Wani *et al*., 2006; Claisse *et al*., 2007; Xia *et al*., 2001). Application of more restriction endonucleases would have increased the taxonomic information of OTU patterns. However, such efforts would have appeared economically questionable, particularly if DNA sequencing techniques were taken into account.

Linearity of the rarefaction curve (Fig.4) suggests that diversity of prokaryotes in activated sludge from SMBR determined experimentally was lower than that from expected theoretically. It was noted that the limited sample sizes and numbers (one clone library) might generate results that did not fully reflect the compositions of the communities, and the coverage value of 82.9% (not too high) also drew the similar conclusion.

The mean removal rates were higher than 80%, and the highest removal rate of COD reached 97.59%, which showed that the performance of SMBR kept high level. Moreover, standard errors of these parameters were quite low, demonstrating the SMBR system remaining stable during this study. For the close relationship between microbial community and performance of MBR, bacterial community compositions should be comparatively stable during this experiment. Unfortunately, it is difficult to adequately disclose the correlation for the limit of insufficient information from this study. Furthermore, due to most of the sequenced clones represented novel taxa we knew few, more targeted searches in some bacterial groups need to be carried out.

4 Conclusions

In summary, bacterial community compositions in activated sludge from SMBR were assessed by constructing full-length sequenced 16S rDNA. Bacterial communities were diversified at the taxonomic level. A number of clones distantly related to sequences from GenBank were potentially sludge related. An overall high richness and evenness implied that larger sample sizes in clone libraries or more targeted searches in some bacterial groups in activated sludge need to be carried out.

Nucleotide sequence accession numbers

The nucleotide sequence data reported in this study have been deposited in the NCBI nucleotide databases under the accession numbers EU283346 to EU283409.

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