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Composition of Microbial Communities in Industrial Drain Outlets

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454-pyrosequencing technology was employed to investigate the changes in bacterial community composition of three different sewage outfalls along the Ningbo coastline as well as to identify shifts in the composition of communities sampled during different months from the same outfalls. A total of 125,746 16S rRNA gene sequences were obtained from 12 samples. The composition of bacterial assemblages was different in the outfalls examined,Across the 12 samples analyzed (3 sites sampled in 4 separate months), the most predominant phylum was Proteobacteria, followed by Bacteroidetes, Acidobacteria and Firmicutes. While Proteobacteria was the predominant phylum in the 12 samples, variation in the classes of Proteobacteria comprising each assemblage were evident. α-Proteobacteria accounted for about 10% of all Proteobacteria found and exceeded 50% of the total proteobacterial sequences in several samples. Likewise, α-Proteobacteria comprised less than 30% of the total proteobacterial sequences in most samples, but exceeded 80 of the total in several samples. These results suggest that the composition of microbial communities shift in response to seasonal and spatial gradients, which likely reflects variation in the physical and chemical characteristics of the sewage.

Key words: Industrial drain outlet, waste water, 454 pyrosequencing, Microbial community structure.

Increased economic productivity has changed the amount and the quality of sewage outfalls in recent years in China. These changing trends have influenced the water quality of adjacent coastal waters. Ningbo is located in the middle coastline of mainland China and has primary sea waters that include the south coast of Hanzhou Bay, the mouth of the Yong River, Xiangshan port, Sanmen Bay, and the adjacent sea areas of Beilun to Daxie. The results of environmental monitoring indicate that wastewater overflow is common in the sewage draining outfalls (State Oceanic Administration Peoples's Republic of China), events which may introduce pollutants to adjacent marine environments. Pollution of coastal regions threatens the health and productivity of the macro and microbial communities inhabiting these marine ecosystems[13,31,36,39]. Xiangshan port, seated in the northern coastal areas of Zhejaing, is a long and narrow semi-enclosed bay near. The bay is impacted by numerous wastewater outlets. Due to geographical considerations, this bay has little exchange with external sea water. As a result, it has been shown to have a unique microbial diversity that is distinct from that found in open sea water [21,26,38]. While many studies have examined the link between microbial community composition and environment[7,16,19,24,37], such studies that focus on sewage outfalls are much rarer. Most scientific studies to date have focused on the wastewater treatment systems themselves. Compared with

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other pollution waters[40], the components of waste from sewage outfall are much more complicated, containing large types of fatty acid and organic compounds, solids(such as sludge,organic solids and disscolved solids), high concentrated nutrients, heavy metal, etc. In addition, the chemical oxygen demand (COD) is also high.

In this study, the characteristics of the sewage outfall were poor, and the content of various organic pollutants and ammonia nitrogen materials exceeded our criteria. Indeed, the site was classified as a heavily polluted outlet (Ningbo city Ocean and Fishery Bureau). We collected seawater samples from three inland-source industrial sewage outfalls in Ningbo, Zhejiang, a region where large industrial companies are located[34]. 454-pyrosequencing technology was applied to investigate the bacterial diversity associated with these sewage outfalls and the shifts in the composition of these communities over a seasonal cycle.

MATERIALS AND METHODS

Sample collection

All samples were collected from 3 sewage outlets in Ningbo, China in 2011. Details regarding these outlets are given in Figure. 1. The untreated tannery wastewater samples were collected in clean, pre-sterilized containers (capacity 5 L). Samples were collected during different seasons: winter (March), spring (May), summer (August), and autumn (October). For each of the 3 sites, samples collected in March were named S1M3,S2M3, S3M3; samples collected in May were named S1M5, S2M5, S3M5; samples collected in August were named S1M83, S2M8, S3M8; samples collected in October were named S1M10, S2M10, and S3M10. The collected wastewater samples were brought to the laboratory and filtered immediately. Wastewater samples were filtered through 0.2 μm polycarbonate filters (Poretics Products, Livermore, U.S.). Filters containing biomass for DNA extraction were stored at -80°C until further processed.

Genomic DNA extraction, PCR amplification, and amplicon purification

Genomic DNA was extracted from the filters using the Fast DNA SPIN kit for Water DNA extraction kit (Omega Bio-Tek, U.S.). Extracts were subjected to quality control. In particular, the absorbance ratio (260 to 280 nm and 260 to 230 nM) were required to be between 1.80 and 2.0 and higher than 1.70, respectively. Here we applied a set of primers to amplify the 16S rRNA gene of bacterial. Fragments of the 16S rRNA gene amplified from total bacterial consortia DNA were amplified using newly designed primers for the selective amplification of bacterial 16S rRNA genes[48].

Total genomic DNA was used as template and PCR reactions were performed in 20μl volumes containing 2μl 10× Ex Taq Buffer (Mg^{2+} Free), 2 mM $MgCl_2$, 0.2 mM dNTP, 0.4μM each of forward and reverse primers, 0.2 U TaKaRa Ex Taq and 50–100 ng DNA template (TaKaRa, Dalian, China). PCR products were verified using gel electrophoresis (2% w/v). The DNA band of correct size was excised and purified with the PCR Clean-up System (Omega Bio-Tek, U.S.). After equal amounts of replicate PCRs were pooled and prepared for 454 pyrosequencing.

DNA samples were subjected to pyrosequencing using the 454 GS Junior and Titanium platforms (454 Life Sciences-OE Biotech Company, Shanghai, China.). Pyrosequencing was conducted on the Titanium platform (Roche/454 Life Sciences). Emulsion PCR was performed using Roche's protocols[28]. Sequencing results were analyzed with Roche software version 2.5.3.

Sequence analysis

Raw sequence data was sorted based on sample-specific barcode tags. Following sorting, the primer and tag sequences were trimmed. Raw sequences were processed through the Ribosomal Database Project II (RDP-II) pyrosequencing pipeline[12] (http://rdp.cme.msu.edu). First, ambiguous and short sequences with a length less than 150bp were removed. Second, sequences were clustered into operational taxonomic units (OTUs) defined by a 3% distance level (97% sequence identities) using the complete-linkage clustering method. Third, these sequences were assigned to phyla using the RDP-II classifier at a 50% confidence threshold [42]. The sequences were clustered based on the similarities of 97%, 95% and 90%.R(v 2.14.2, http://www.r-project.org/) was used to calculate the diversity indices,Shannon-Wiener index, Simpson's Diversity index and Evenness index.

RESULTS AND DISCUSSION

Microorganisms play a significant role in reducing the impact of sewage discharge into the sea through their activities, which effectively remove numerous nutrients from sewage or transform them to less toxic products[3,5,20,]. Pyrosequencing techniques are high-throughput analytical tools capable of generating large numbers of DNA reads through a massively parallel sequencing-by-synthesis approach[12,15,27]. This technology has seen widespread use in the analysis of microbial communities in various environmental samples[48]including the marine water column[1,33], terrestrial soils[35], human and animal intestinal flora[8,2841,49]and wastewater treatment plant influent[29]. A total of 125,746 sequences were obtained from the 12 samples (3 different sites sampled in 4 separate months) analyzed. The heat map shows taxonomic prevalence of bacterial phyla among three sewage outfalls sampled from different months (Figure 2.). At a broad level, no clear pattern of assemblage clustering was observed based on sample location or based on sampling data. Two primary clusters were evident, one of which was comprised of samples S1M3, S2M5, S2M10, S3M3, S3M5 and S3M8 and the other which was comprised of S2M8, S1M10, S1M8, S2M3, S3M10, and S1M5.

The bacterial communities associated with 3 different sewage outfalls differed at the phylum level of taxonomic composition. Sequences affiliated with Proteobacteria were the most abundant followed by those affiliated with

Table 1. Sequencing and diversity statistics associated with bacterial 16S rRNA gene libraries obtained from samples

Sample name	Reads[a]	Shannon-Wiener[b]	Simpson's Diversity[c]	Evenness[d]
S1M3,	16254,	4.33,	0.82,	0.69
S1M5,	15301,	4.02,	0.76,	0.66
S1M8,	5601,	4.51,	0.81,	0.74
S1M10,	9785,	5.33,	0.88,	0.79
S2M3,	7356,	4.82,	0.80,	0.66
S2M5,	20044,	5.11,	0.84,	0.71
S2M8,	5342,	6.56,	0.90,	0.82
S2M10,	8672,	3.03,	0.76,	0.56
S3M3,	16635,	4.22,	0.81,	0.62
S3M5,	10060,	5.46,	0.88,	0.75
S3M8,	5693,	4.21,	0.87,	0.69
S3M10,	5297,	6.21,	0.91,	0.72

a,Good reads is the quality control passed reads more than 150 bp.
b,A higher number represents more diversity.
c,A higher number represents more diversity.
d,A higher number represents more evenness.

Firmicutes and Bacteroidetes. In sewage outfall S1 (Fig. 3a.) the relative abundance of Proteobacteria changing little in March and May from 86% to 89% of the total sequences obtained. However, the relative abundance of sequences affiliated with Proteobacteria decreased to 79% in August and 53% of the total in October. In addition, the relative abundance of sequences affiliated with Bacteroidetes was less than 3% of the total on March and May but increased to 15% and 33% of the total in August and October, respectively. Furthermore, the abundance of Firmicutes was from less than 2% of the total sequences in March, August and October but was 8% of the total sequences in May.

In S2 (Fig. 3b.), the abundance of sequences affiliated with Proteobacteria was highest (96% of total sequences) in October. However, in March the percentage of sequences affiliated with Proteobacteria was 50% of total sequences, compared to only , 42% and 5% of total sequences for Bacteroidetes and Firmicutes. The abundance of sequences affiliated with Proteobacteria in May was roughly 82% in May,

with sequences affiliated with Bacteroidetes represented roughly 11%. Compared with the other three months, in August the content of microflora was more complex, with sequences affiliated with the Proteobacteria (55% of total), Bacteroidetes (13% of total), and Cyanobacteria (10% of total). In addition, the abundance of sequences affiliated with Actinobacteria was 10%, but in the other months, it is only 1%. The composition of Proteobacteria, when classified as the class level of taxonomic, is generally the same in May and August, with sequences affiliated with α-proteobacteria, β-proteobacteria, and γ-proteobacteria representing 54%, 6%, and 35% of the total proteobacterial sequences, respectively. In May and October, sequences affiliated with ^{3}-proteobacteria represented 54% and 46% of the total proteobacterial sequences, respectively (Fig. 3d.).

In S3 (Fig. 3c.), sequences affiliated with Proteobacteria are the most abundant in March, May and August while only representing 37% in October. Sequences affiliated with Cyanobacteria represented 37% of the total in October, but were less than 2% of the total sequences in March, May and October. The proteobacterial assemblages were dominated by sequences affiliated with the ^{3}-proteobacteria in March (64% of total sequences) and October (71% of total sequences). Sequences affiliated with α-proteobacteria represented the largest fraction of the proteobacterial assemblage in August (Figure 3d.).

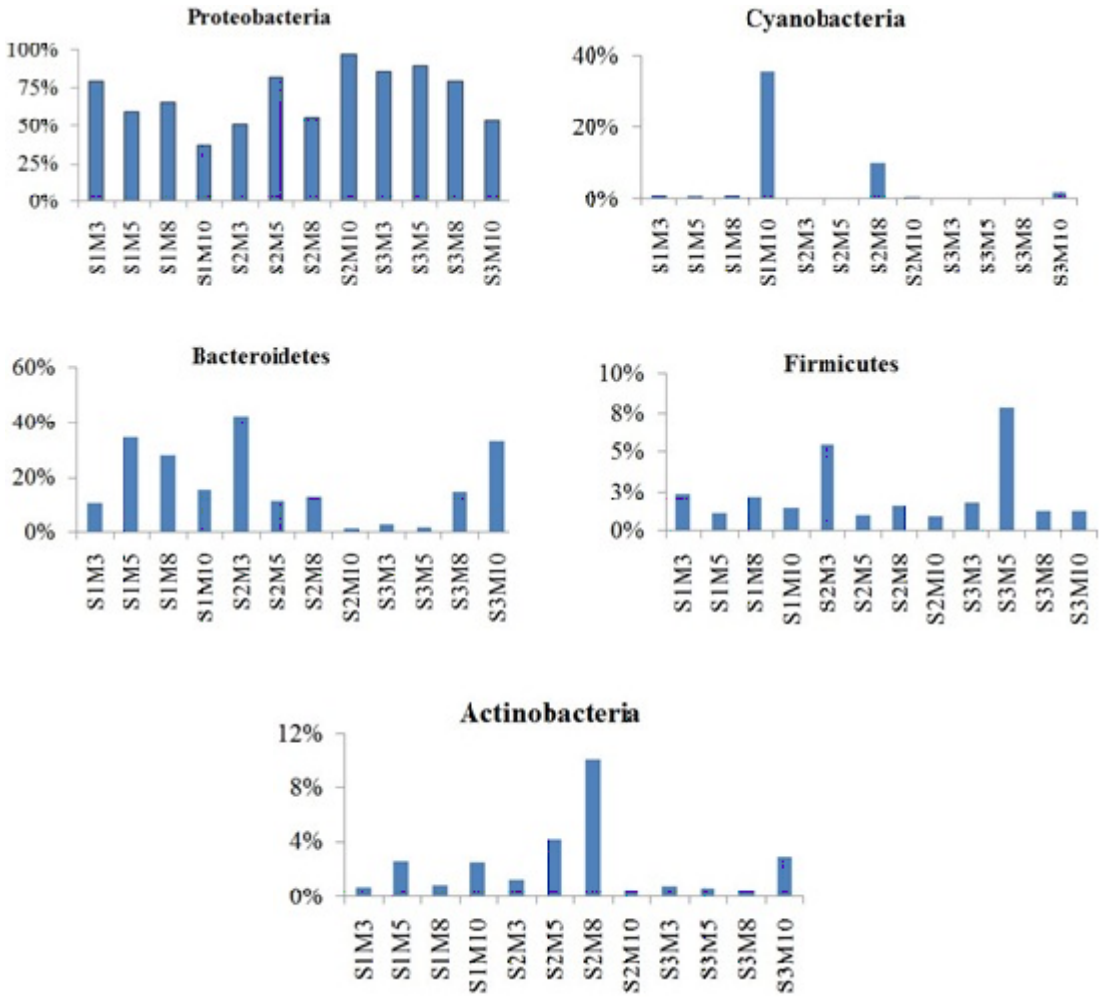

Fig. 1. Map depicting location of sampling sites

Fig. 2. Dendogram depicting the similarity in the taxonomic (OTU-based) composition of bacterial assemblages. Relative abundance of sequences associated with each sample is indice samples. Each row represents the phylum level taxonomic affiliations. Color intensity for all panels indicates the relative abundance of the microbial descriptions within each sample, with the legend indicated at the upper left corner. 'Other' refers to the taxa with a maximum abundance of <1% in any sample

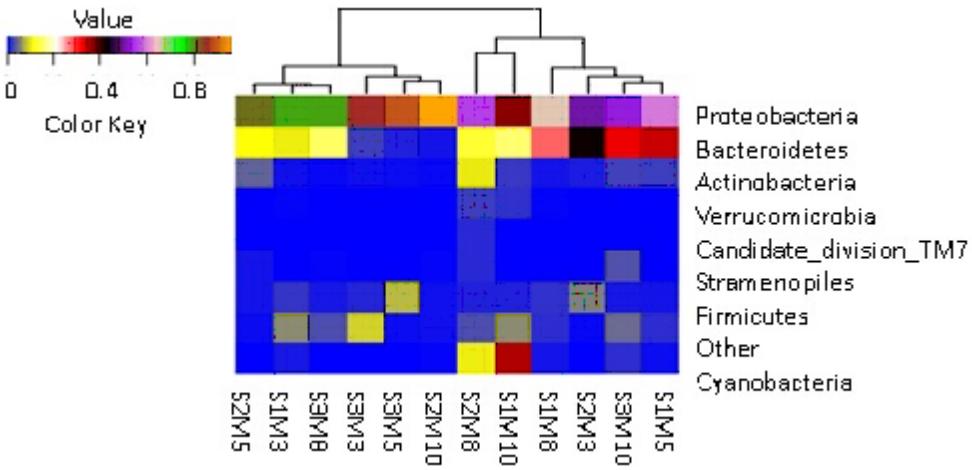

Fig. 3. Taxonomic composition, as depicted by phylum level classification, of bacterial communities associated with different wastewater. Other refers to sequences that binned to phyla that represented <1% of the total sequences. a: bacterial communities of sewage outfall S1;b: bacterial communities of sewage outfall S2; c: bacterial communities of sewage outfall S3;d:Relative abundance of sequences identified as Proteobacterial when binned at a class level taxonomic rank

Proteobacteria and Bacteroidetes are ubiquitous in environment [17,43,44].Thus, these results suggest terrigenous microbe discharge to the coastal waters via sewage. While other ecosystems contain a high abundance of Firmicutes[22,30,46], sewage outfalls tended to not have a significant abundance of firmicuts. Previous reports suggested that microcolonies of Firmicutes were weak and cannot resist strong shear imposed on them, unlike Proteobacteria[25,46]. While Proteobacteria was the predominant phylum in the 3 samples(Figure 4.), variation in the dominant class of Proteobacteria present were evident between samples. α-Proteobacteria was dominant in the marine microbial community which were found at the surface of sea water [2,50]. In the 12 samples characterized here, α-Proteobacteria accounted for about 10% of all Proteobacteria identified. In some samples (i.e., S2M5 and S2M8), the percentage of α-Proteobacteria exceeded 50% in S2M5 and S2M8, which was not common in wastewater treatment plants'[18]. β-Proteobacteria is uncommon in seawater samples[2]. Nonetheless, in our study, sequences affiliated with the β-Proteobacteria were abundant and their abundance shifted over the time course of this study. For example, β-Proteobacteria represented 80% of the proteobacterial assemblage in S1M3 but represented less than 30% in the rest samples. The large abundance of Proteobacteria may be due to the original percolating water in March containing large quantities of β-Proteobacteria or may be due to the influence of some materials in percolating water, which can stimulate the production of β-Proteobacteria. The primary γ-Proteobacteria taxa identified included Enterobacteraceae, Vibrionacea, Pseudomonadaceae, Salmonella, Yersinia, Vibrio, and Pseudomonas aeruginosa, many of which are common in wastewaters and soils[21,26,38]. Moreover, a number of sequences affiliated with potentially pathogenic bacteria (e.g., *P. aeroginosa*) were identified in some samples. These results suggest that under the stimulation of some materials present in waste water, certain pathogenic bacteria may have been produced and discharged into seawater together with the sewage, which is likely to significant affect the marine environment and marine life. Indeed, recent reports indicate the sea farming near the sewage outlets has low yields with significant death rates. Further study needed to see if it is related with the mass propagation of the pathogenic bacteria.

Bacteroidetes is another most abundant phyla in the samples analyzed (Fig. 4). According to the Ningbo Marine Environment Bulletin (Ningbo city Ocean and Fishery Bureau, China),

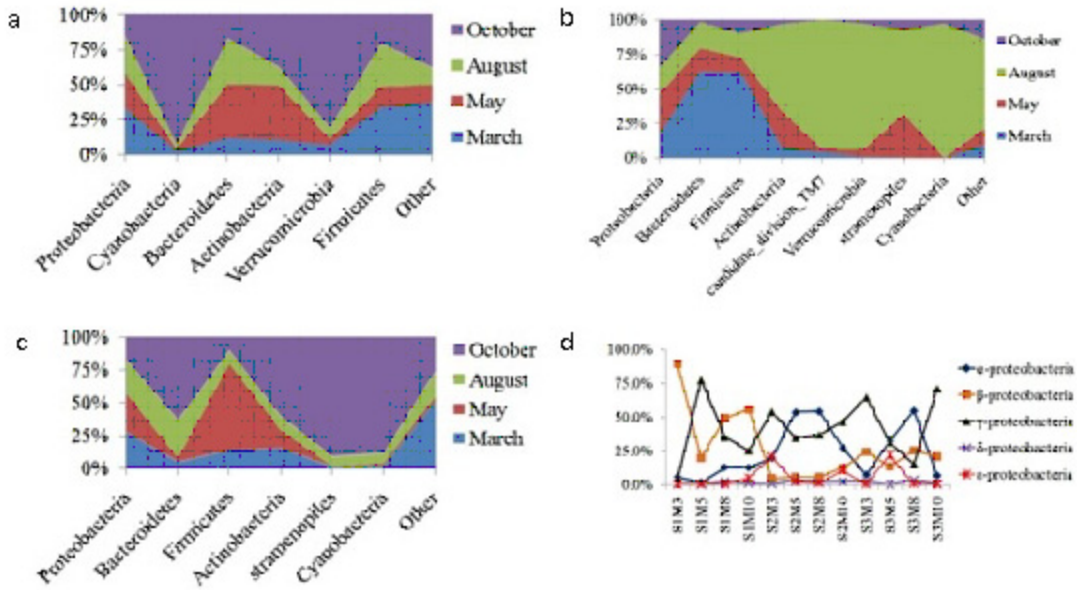

Fig. 4. Relative abundance of Proteobacteria, Cyanobacteria, Bacteroidetes, Firmicutes and Actinobacteria in different samples

the contents of COD, ammonia nitrogen, and volatile phenol in the samples we collected in this study exceed standard. Cyanobacteria (Figure 4) were abundant in several samples (e.g., S2M8 (10%) and S3M10 (36%)), which may be related to the high concentration of ammonia nitrogen. Mass propagation of Cyanobacteria has been shown to cause the deterioration of water quality and more seriously cause fish death by exhausting the oxygen in water[4,6,10,14,32].

Many Firmicutes can produce endospores, which can resist dehydration and extreme environmental conditions[18,24]. They are found in various environments, and the group includes some notable pathogens. In most samples, the relative abundance of Firmicutes was lower than 3%, but in S2M3 and S3M5 its percentage was over 5%. Environmental factors, such as pH, phosphate levels, and COD play an important role in Microbial Communities[9,25].Due to the diversity of industrial production, the categories of pollutant ingredients were quite different. According to the statistical analysis performed by the Ningbo City Ocean and Fishery Bureau, environmental conditions in S2M3 and S3M5 were relatively poorer than other samples, which may account for the percentage increase of Firmicutes. Besides these, the percentage change of Cyanobacteria (Fig. 3) was prominent, in S1M10 and S2M8 its percentage were 36% and 10%, while in other samples its percentage was lower than 2%, in some samples its percentage even broke down to 1%, analysis of its reason may also result to the influence of environmental factors.

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REFERENCES

1. Angly, F.E., B.Felts , M. Breitbart, P. Salamon, R.A. Edwards, C. Carlson, A.M. Chan; M. Haynes, S.Kelley, L.Hong, J.M. Mahaffy, J.E. Mueller,; J.Nulton, R.Olson, R.Parsons, S. Rayhawk, C.A. Suttlev and F.Rohwer. The marine viromes of four oceanic regions. *PLoS Biol.*, 2006; **4**: e368.
2. Bai,J., H.Y. Li and Y.G. Zhao. Bacterial distribution at different stations in the Northern Yellow Sea. *Acta Microbiol Sin.*, 2009; **49**(3): 343-350.
3. Bai,J., H.F. Zhang, K.R. Li, J. Zhang, D.Y. Liu, D.M. Gao,Q.T. Yial. Distribution and relationship between bacterioplankton and inorganic nutrients in Jiaozhou Bay in winter. *Mar Sci.*, 2004; **28**(12): 31-34.
4. Beattie,K.A., K.Kaya, T.Sano and G.A.Codd. Three dehydrobutyrine (Dhb)-containing microcystins from the cyanobacterium *Nostoc sp.Phytochemistry*, 1998; **47**: 1289-1292.
5. Cao,G.M., Q.X. Zhao, X.B. Sun and T. Zhang. Integrated nitrogen removal in a shell-and-tube co-immobilized cell bioreactor. *Process Biochem*., 2004; **39**(10): 1269-1273.
6. Carbis,C.R., G.T. Rawlin, P. Grant, G. F. Mitchell, J.W.Anderson and I. McCauley. A study of feral carp, Cyprinus carpio L., exposed to Microcystis aeruginosa at Lake Mokoan, Australia, and possible implications for fish health.*J. fish dis*., 1997; **20**: 81-91.
7. Cetecioglu,Z., B.K. Ince, M. Kolukirik and O.Ince. Biogeographical distribution and diversity of bacterial and archaeal communities within highly polluted anoxic marine sediments from the marmara sea. *Mar Pollut Bull*., 2009; **58**(3): 384-395.
8. Claesson, M., O. O'Sullivan, Q. Wang, J. Nikkila, J.R. Marchesi, H. Smidt, W.M. de Vos R.P.Ross and P.W. O'Tool .Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. *PloS One* , 2009; 4e6669.
9. Chandra,R., R.N. Bharagava, A. Kapley and H.J. Purohit. Bacterial diversity, organic pollutants and their metabolites in two aeration lagoons of common effluent treatment plant (CETP) during the degradation and detoxification of tannery wastewater. *Bioresource Technol.*, 2011; **102**: 2333-2341.
10. Chen,J. and P. Xie. Tissue distributions and seasonal dynamics of the hepatotoxic microcystins-LR and -RR in two freshwater shrimps, Palaemon modestus and Macrobrachium nipponensis, from a large shallow, eutrophic lake of the subtropical China. *Toxicon*., 2005;**45**: 615-625.

11. Cole,J.R., Q. Wang, E. Cardenas, J. Fish, B. Chai, R.J. Farris, A.S. Kulam-Syed-Mohideen, D.M.McGarrell, T.Marsh , G.M.Garrity and J.M.Tiedje. The ribosomal database project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Res.*, 2009; **37**: D141-5.
12. DeAngelis,K.M., C.H Wu, H.R. Beller, E.L. Brodie.Chakraborty R., T.Z. DeSantis, J.L. Fortney, T.C.Hazen, S.R.Osman, M.E.Singer, L.M.Tom and G.L. Andersen.PCR amplification-independent methods for detection of microbial communities by the high-density microarray phylochip. *Appl Environ Microbiol.* 2011; **77** (18): 6313-6322.
13. Dalby, P.D., K.A.Kormas, U. Christaki and H. Karayanni. Cosmopolitan heterotrophic microeukaryotes are active bacterial grazers in experimental oilpolluted systems. *Enviro Microbiol.*, 2007; **10**: 47-56.
14. Dong,G.F., S.Q. Xie, X.M. Zhu, D. Han and Y.X.Yang. Nutri-toxicological effects of cyanobacteria on fish. *Acta Ecologica Sinica.*, 2012; **32**(19): 6233-6241.
15. Fabrice,A. and R. Didier.Exploring microbial diversity using 16S rRNA high-throughput methods. *J.Comput. Sci. Syst. Biol.* 2009; **2**: 74-92.
16. Feng,B.W., X.R Li., J.H. Wang, Z.Y. Hu, H. Meng, L.Y. Xiang, Z.X.Quan. Bacterial diversity of water and sediment in the Changjiang estuary and coastal area of the East China Sea. *FEMS Microbiol Ecol.*, 2009; **70** (2): 236-248.
17. Fierer, N. M.A. Bradford, R.B. Jackson. Toward an ecological classification of soil bacteria. *Ecology.*, 2007; **88** (6):1354-1364.
18. Hu,M., X.H. Wang, X.H. Wen and Y.Xia. Microbial community structures in different wastewater treatment plants as revealed by 454-pyrosequencing analysis. *Bioresource Technol.*, 2012; **117**: 72-79.
19. Ikenaga,M.,R.Guevara, A. Dean, C. Pisani, and J.N Boyer. Changes in community structure of sediment bacteria along the Florida coastal everglades marsh-mangroveseagrass salinity gradient. *Microbial Ecol.*,2010; **59**(2): 284-295.
20. Jia,P. and X.Deng. Progress in the application of photosynthetic bacteria to the treatment of heavy metal wastewater.*Industrial Water Treatment*, 2011; **31**(1): 13-17.
21. Kent,K.C.B, M.K. David and W.C. Sallie. Nutrient gradients in the western North Atlantic Ocean: Relationship to microbial community structure and comparison to patterns in the Pacific Ocean. *Deep-Sea Res PT I*, 2001; **48**(11): 2373-2395.
22. Kwon, S., T.S. Kim, G.H. Yu, J.H. Jung and H.D.Park.,Bacterial community composition and diversity of a full-scale integrated fixed-film activated sludge system as investigated by pyrosequencing. *J. Microbiol. Biotechnol*, 2010; **20** (12): 1717-1723.
23. Larsen, P., J.L. Nielsen, T.C. Svendsen and P.H.Nielsen. Adhesion characteristics of nitrifying bacteria in activated sludge.*Water Res.*, 2008; **42**(10-11): 2814-2826.
24. Li,J.L., Z.H Wang, S. Qin and G.Y. Wang. Microbial diversity of sediments from the coasts of Dalian Changshan Islands.*Acta Microbiol Sin.*, 2011; **51**(5): 656-666.
25. Li,J.M.and Z.X.Jin. Effect of hypersaline aniline-containing pharmaceutical wastewater on the structure of activated sludge-derived bacterial community. *J.Hazard Mater.,* 2009; **172**: 432-438.
26. Li,Z.Y., L.M. He , J. Wu and J.Qun. Bacterial community diversity associated with four marine sponges from the South China Sea based on 16S rDNA-DGGE fingerprinting. *J Exp Mar Biol Ecol.*, 2006; **329**(1,7): 75-85.
27. Margulies, M., M. Egholm, W.E. Altman, S. Attiya, J.S. Bader, L.A. Bemben, J.Berka, M.S.Braverman, Y.J.Chen, Z.Chen, S.B.Dewell, L. Du, J.M.Fierro, X.V.Gomes, B.C.Godwin, W. He, S.Helgesen, C.H.Ho, G.P.Irzyk, S.C. Jando, M.L .Alenquer, T.P.Jarvie, K.B. Jirage, J.B.Kim, J.R.Knight, J.R.Lanza, J.H. Leamon, S.M. Lefkowitz, M.Lei, J.Li, K.L.Lohman, H.Lu, V.B.Makhijani, K.E. McDade, M.P.McKenna, E.W.Myers, E.Nickerson, J.R.Nobile, R.Plant, B.P.Puc, M.T.Ronan, G.T.Roth, G.J. Sarkis, J.F.Simons, J.W.Simpson, M.Srinivasan, K.R. Tartaro, A.Tomasz, K.A.Vogt, G.A.Volkmer, S.H.Wang, Y.Wang, M.P.Weiner, P. Yu, R.F.Begley and J.M. Rothberg.Genome sequencing in microfabricated high-density picolitre reactors. *Nature*, 2005; **437**(7057): 376-380.
28. McKenna,P., C.Hoffmann, N.Minkah, P.P.Aye, A. Lackner, Z.Liu, C.A.Lozupone, M. Hamady, R. Knight, F.D.Bushman. Themacaque gut microbiome in health, lentiviral infection, and chronic enterocolitis.*PLoS Pathog*, 2008; **4**(2): e20.
29. McLellan,S.L., S.M.Huse, S.R.Mueller-Spitz, E.N.Andreishcheva M.L. Sogin. Diversity and population structure of sewage derived microorganisms in wastewater treatment plant influent. *Environ Microbiol.*, 2010;**12**:378-392.
30. Oerther,D.B., R.Delos, M.F. Raskin, L. Raskin. Quantifying filamentous microorganisms in

activated sludge before, during, and after an incident of foaming by oligonucleotide probe hybridizations and antibody staining. *Water Res*., 2001; **14**: 3325-3336.

31. Ogilvie, L.A. and A. Grant. Linking pollution induced community to lerance(PICT) and microbial community composition in chronically metal polluted estuarine sediments. *Mar Environ Res*., 2008; **65**: 187-198.
32. Ozawa,K.and H.D.Park. Accumulation and depuration of microcystin produced by cyanobacteria Microcystis in freshwater snail.*Limnol*, 2003; **4**: 131-138.
33. Qian, P., Y. Wang, O. Lee, S.C. Lau, J. Yang, F.F. Lafi, Al-Suwailem. A, and T.Y.Wong. Vertical stratification of microbial communities in the Red Sea revealed by 16S rDNA pyrosequencing. *ISME J*., 2011; **5**: 507-518.
34. Qiu,Q.F., D.M. Zhang, X.S. Ye and J.Qun. The bacterial community of coastal sediments influenced by cage culture in Xiangshan Bay, Zhejiang, China. *Acta Ecologica Sinica*., 2013; **33**(2): 483-491.
35. Roesch,L.F., Fulthorpe R.R., Riva A., Casella G., Hadwin A.K., Kent A.D., S.H. Daroub, F.A.Camargo, W.G.Farmerie, E.W.Triplett. Pyrosequencing enumerates and contrasts soil microbial diversity. *ISME J*., 2007; **1**(4): 283-290.
36. Sauret,C., U. Christaki, P. Moutsaki, I. Hatzianestis, A. Gogou and J.F.Ghiglione. Influence of pollution history on the response of coastal bacterial and nanoeukaryote communities to crude oil and biostimulation assays. *Mar Environ Res*, 2012; **79**:70-78.
37. Sahan,E. and G. Muyzer. Diversity and spatio-temporal distribution of ammonia-oxidizing Archaea and Bacteria in sediments of the Westerschelde estuary. *FEMS Microbiol Ecol*, 2008; **64**(2):175-186.
38. Schnetzer,A., S.D. Moorthi, P.D.Countway, R. J, Gastc, I. C. Gilgd and D.A. Caron. Depth matters: Microbial eukaryote diversity and community structure in the eastern North Pacific revealed through environmental gene libraries. *Deep-Sea Res PtI.*, 2011; **58**(1): 16-26.
39. Song,L.R. and W. Chen. Production of microcystins in bloom-forming cyanobacteria and their environmental. *Journal of Lake Science*, 2009; **21**(6): 749-757.
40. Thiyagarajan,V., M.M.Y. Tsoi, W. Zhang and P.Y. Qian. Temporal variation of coastal surface sediment bacterial communities along an environmental pollution gradient. *Mar Environ Res*, 2010; **70**: 56-64.
41. Turnbaugh,P.J., R.E. Ley, M.A. Mahowald, V. Magrini, E.R. Mardis and J.I. Gordon. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature,* 2006; **444**(7122): 1027-31.
42. Wang Q., G.M. Garrity, J.M. Tiedje and J.R. Cole. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol*, 2007; **73**: 5261-7.
43. Wang,J.L.and C. Chen. Biosorbents for heavy metals removal and their future. *Biotechnol Adv*, 2009; **27**(2):195 -226.
44. Wei,M.M.,R.B.Zhang, Y.G. Wang, H.G. Ji , J. Zheng, X.H. Chen, H.B.Zhou. Microbial community structure and diversity in deep-sea hydrothermal vent sediments along the Eastern Lau Spreading Centre.*Acta Oceanol Sin.*, 2013; **32**(2): 42-51.
45. Wilen, B.M., M. Onuki, M.Hermansson, D.Lumley,T.Minoa. Microbial community structure in activated sludge floc analysed by fluorescence in situ hybridization and its relation to floc stability.*Water Res.*, 2008; **42**(8-9): 2300-2308.
46. Xia,S., L.Duan, Y. Song, J. Li, Y.M. Piceno, G.L. Andersen, L.Alvarez-Cohen, I.Moreno-Andrade, C.L. Huang and S.W.Hermanowicz. Bacterial community structure in geographically distributed biological wastewater treatment reactors. *Environ Sci Technol.*, 2010; **44**(19): 7391-7396.
47. Yang,C., C. Hamela, Y.T. Gana and V. Vujanovic. Tag-encoded pyrosequencing analysis of the effects of fungicide application and plant genotype on rhizobacterial communities.*Appl Soil Ecol.*,2012; **60**: 92-97.
48. Ye,L., M.F. Shao, T. Zhang, A.H. Tong and S. Lok. Analysis of the bacterial community in a laboratory-scale nitrification reactor and a wastewater treatment plant by 454-pyrosequencing. *Water Res.*, 2011; **45**: 4390-4398.
49. Zhang, C., G.M. Zhang, S. Wang, R.Han, Y. Cao, W. Hua,Y. Mao,X. Zhang, X. Pang,C., Zhao G, Y. Chen and L Zhao. Interactionsbetween gutmicrobiota, hostgenetics and diet relevant to development of metabolic syndromes inmice. *ISME J.*, 2009; **4**(2):232-241.