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Factors associated with the diversification of the microbial communities within different natural and artificial saline environments

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ABSTRACT

To investigate whether there is a core microbial community of natural and industrial saline environments and to evaluate the effects of salinity on microbial communities, two natural samples and four artificial samples taken from different locations in China were analyzed by constructing 16S rRNA gene cloning libraries. The results showed that natural samples have a significantly higher halotolerant and halophilic bacterial diversity compared to artificial samples. In total, 9 phyla were detected among the samples, but only *Bacteroidetes* and *Proteobacteria* were commonly shared. The canonical correspondence analysis demonstrated that the community variance correlated strongly with Cl^-, DOC, conductivity, and pH. Moreover, the Q-PCR results revealed that salinity influenced the microbial community structure and progressively decreased the populations of bacteria, archaea, fungi and yeast. Variation partitioning analysis illustrated that DOC/Cl^- had the greatest contribution to bacterial population variation, revealing a core control factor used for functional resilience in saline wastewater treatment applications.

1. Introduction

Salt (mainly NaCl) plays an important role in the ecological system in both natural and artificial environments. Sodium is the principal cation of extracellular fluid. It plays vital and diverse roles in microbial organisms, including the maintenance of the extracellular fluid volume and water balance and the generation of the membrane potential of cells. However, throughout the world, salt consumption has increased, resulting in a large amount of saline effluent from artificial factories, causing a serious environmental problem. Meanwhile, ocean pollution has also become a serious problem due to large amounts of organic wastewater (Weisberg, 2011). Many industrial sectors can generate highly saline wastewater, such as the food-processing industry, leather industry, and petroleum industry (Lefebvre and Moletta, 2006). Moreover, soil salinity, as an increasingly important process of land degradation, is a major threat to microbial communities and thus strongly alters organic matter turnover processes (Tejada et al., 2006).

Faced with tightening regulations, the interest in saline effluent treatment processes, both for salt and organic matter removal, has been increasing rapidly over the last 10 years (Gebauer, 2004; Shi et al., 2014; Taheri et al., 2012). However, during the biological treatment of wastewater, an increase in salt concentration results in a reduction of the biodegradation rate. Furthermore, the salt content in wastewater also reduces the population of organisms, resulting in a lower treatment efficiency (Lefebvre and Moletta, 2006). Previous research has characterized halophilic heterotrophic nitrification, which has an aerobic denitrification ability at high salinity (Duan et al., 2015). In addition, even the interactions of operation factors were investigated in saline wastewater treatment (Sudarno et al., 2011). Previously conducted An/Aerobic biotreatment processes demonstrated that greater than 5–8 g Cl^-/L in the traditional aerobic treatment will cause inhibition and a sodium concentration exceeding 10 g/L strongly inhibits anaerobic methanogens (Kugelman and McCarty, 1965). Increasing salinity thus has detrimental effects on biological mediated processes. The adaptation of an active methanogen at the salinity level of the effluent was shown to be possible, and the authors concluded that the efficiency of such a process depended on a suitable strategy for adapting the biomass to high salinity. The ability to resist high salinity stress is essential to the microbial community.

The ability to resist high salinity stress is the core of constructing a suitable microbial community to achieve an efficient

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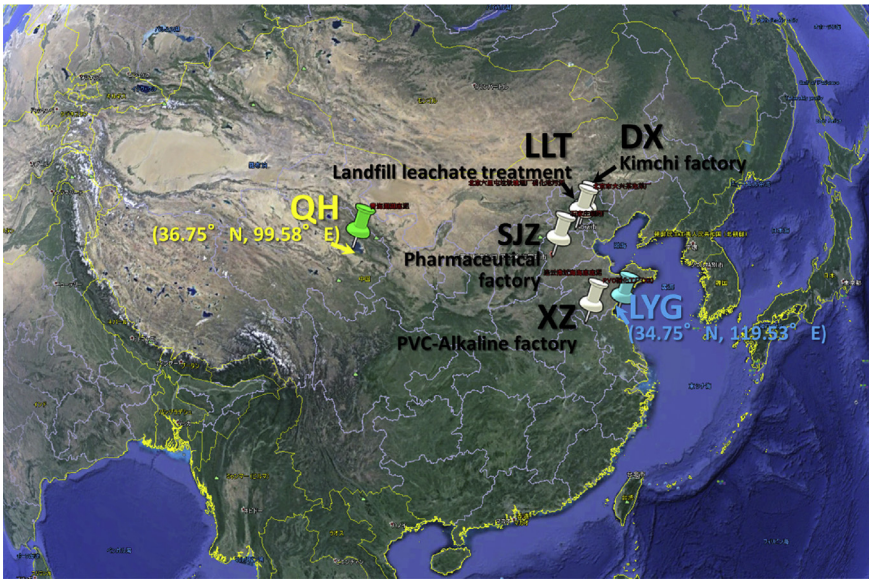

Fig. 1. Sampling locations of artificial and natural saline environment in China.

bio-treatment process. Therefore, well-adapted seed sludge is the key step for establishing a reactor. Knowing the microbial community structure of the salt environment is important because community diversity and functional group abundance can shape the dominate group, and the major halotolerant and halophilic bacteria are naturally resistant to environmental pressure variations. Additionally, a fast start up and highly efficient performance is valuable for engineering applications. Thus, the purpose of this study was to investigate whether there is a core microbial community in the microbial populations of different natural and industrial saline environments and to evaluate the effects of salinity water characteristics and artificial effects on microbial communities.

2. Material and methods

2.1. Location and characterization of the samples

Different sludge samples were collected from natural saline environments: LYG (34.75°N, 119.53°E) sediment from the Lianyungang marine and QH (36.75°N, 99.58°E) sediment from Qinghai saline lake. The artificial saline samples were taken from four different types of factories located in China (Fig. 1): a kimchi factory (DX), Liulitun landfill leachate (LLT), pharmaceutical factory (SJZ) and Xuzhou PVC–alkaline factory.

2.2. 16S rRNA gene cloning library construction

Samples were washed 3 times with PBS. Microbial DNA was directly extracted from the samples with a Fast DNA SPIN Kit for Soil (MP Biomedicals LLC) according to the manufacturer's instructions. The isolated DNA was stored in a refrigerator at −20 °C.

The microbial community was analyzed by 16S rRNA gene cloning and sequencing. The amplification of 16S rRNA gene cloning was performed with the primers 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT) for bacteria (Lane, 1991). The PCR was performed in a final volume of 50.0 μL and included 10 × PCR buffer (1.5 mmol/L $MgCl_2$), 5.0 μL; dNTP (2.5 mmol/mL), 4.0 μL; each primer (10 μmol/L), 1.0 μL; Taq DNA (2.5 μ/μL), 0.5 μL; template DNA, 1.0 μL; and sterile distilled water, 37.5 μL. The amplification program was: 94 °C for 5.0 min, 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1.5 min, and a final extension at 72 °C for 7.0 min. Five microliters of the amplified mixture was analyzed by 1.5% 0.5 × TBE agarose gel electrophoresis. The gel was stained with ethidium bromide, visualized under UV light and photographed. Amplified 16S rRNA genes were purified with the Qiaquick PCR cleanup kit (Qiagen Inc., Chatsworth, CA). The cloning of amplified 16S rRNA gene fragments into the pMD-18-T vector and the selection of TOP10 *Escherichia coli* transformants were performed following the manufacturer's instructions (Invitrogen, Shanghai, China). The cloned inserts were amplified from lysed colonies with vector-specific primers M13F (5′-GTAAAACGACGGCCAG-3′) and M13R (5′-CAGGAAACAGCTATGAC-3′). The PCR products were digested (3.0 h, 37 °C) with Hae III (Takara), separated by electrophoresis in 1.5% 0.5 × TBE agarose gels, and then grouped according to restriction fragment length polymorphism (RFLP) patterns. Representative clones were sequenced by the Beijing AuGCT DNA-SYN Biotechnology Company. A similarity search was performed using the NCBI Blast search program within the GenBank database (http://www.ncbi.nlm.nih.gov/blast/). Sequences obtained in this study have been deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases with the following accession numbers: nature samples, HQ703793–HQ703888, and artificial samples, HQ738353–HQ738469. A Bio-systems 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA) was used for Q-PCR. SYBR® green I (Takara, Dalian, China) was used as a double-stranded DNA (dsDNA) binding dye, whereby the fluorescence intensity of the dye increased with the amount of amplified dsDNA. Each reaction mixture (20 μL) was composed of 10 μL of SYBR® *Premix Ex Taq*™ (2×), each of the specific primers (10 μmol), 0.4 μL; ROX Reference Dye (50×), 0.4 μL; template RNA, 2.0 μL; and sterile distilled water, 6.8 μL. The real time-PCR measurements were repeated at least twice. The primers are listed in Table 1.

All of the sequences were grouped into operational taxonomic units (OTUs) using Mothur (Schloss et al., 2009). Based on the grouping of unique OTUs, the frequency data for each distance level was used to construct rarefaction curves and the diversity indices. The richness index of Chao, Coverage and Shannoneven was also calculated to assay the cloning library and microbial diversity.

2.3. Analytical methods

Samples taken from the salty environment were frequently analyzed. PHS-2F (Shanghai, China) was used for pH analyses. The