

Microbial biodiversity in Bohai Bay Saltworks, China and their biotechnological utilization

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1. INTRODUCTION

The multi-pond solar saltworks consist of a series of conjunctive saltponds, with a gradient of salinities ranging from seawater to NaCl precipitation. Solar salterns inhibit diverse microbial groups. Along the salinity gradient, the majority of microflora changes from marine origins to moderately halophiles, and extremely halophiles (Antón et al., 2000; Oren, 2002). In addition to being part of the food chain in hypersaline ecosystem (Toi et al., 2013; 2014), halophilic microorganisms also play an important role in water quality management such as reducing the nitrogenous elements and viscosity of brine water, through nitrification, ammonium assimilation and oxidation of organic matters. In crystallization ponds, the blooms of red halophilic bacteria and archaea ensure the increased heat absorption, ultimately resulting in an enhanced evaporation and an improved salt crystallization (Jones et al., 1981).

Apart from the ecological importance, the use of halophiles in biotechnology has been recently concerned. Halophilic bacteria and archaea are useful biological sources to produce bio-active compounds such as polyalkanoate, ectoine, extracellular polysaccharides and carotenoid pigments (Oren, 2002). Moreover hypersaline living conditions (usually >20% NaCl) are beneficial to the large scale production with less danger of contamination, and thus facilitate the industrial application of the halophilic microorganisms.

Bohai Bay coast is the major sea salt producing area in China, with dozens of saltworks covering 1500 km² and a total annual yield of 20 million tons (data in 2012, China Salt Association). Even though being an important salt production site, the ecological study of the salt ponds, particularly the microbial diversity and their biotechnological exploration have not yet been characterized.

Recently we have studied the variation of microbial biodiversity corresponding with different salinities and seasons in Bohai Bay saltworks. A number of cultivable bacterial and archaeal strains were isolated and characterized from salt ponds. Moreover, the accumulation of biotechnological compounds such as poly- β -hydroxybutyrate and

bacterioruberin were studied with specific strains. The aim of our studies is to provide useful information on the potential utilization of microbial resources in saltern ponds.

2. BACTERIAL BIODIVERSITY IN SALT PONDS

The bacterial diversity of brine water in different salt ponds and seasons in Hangu Saltworks were analyzed using PCR-DGGE method. The salinity of five sampled salt ponds ranges from 60 to 180, with an average difference of salinity 30 among each other. The average water temperature in May, July and October were 20°C, 28°C and 16°C, respectively. The phylogenetic analysis on DGGE bands showed that the temperature had more effect on the bacterial community than the salinity. Corresponding with the complex band patterns observed on the DGGE gel, the range-weighted richness (Rr) were mostly higher than 30, indicating that the investigated saltponds were habitable environments with broad carrying capacity (Marzorati et al., 2008). Moreover Shannon-Wiener's index in July (2.6-3.1) was generally higher than that of May (2.5-2.9) and October (2.6-2.9), and within the same month higher salinity usually resulted in a lower value, showing that the biodiversity of brine water decreased with increasing water temperature and salinities (Ma et al., 2014).

16S rRNA gene sequencing of DGGE bands revealed that, out of the 48 sequenced bands, 15 bands related to γ -proteobacteria, 6 bands to Bacillaceae, 2 bands to Bacteroidetes, 3 bands to Flavobacteriaceae, 1 band to Rhodothermaceae, 1 band to Micrococcineae and 1 band to α -proteobacteria, with similarity of 83%-100%. Most of sequences originated from salterns or saline environments, whilst a few related to the sequences from marine environment (Ma et al., 2014).

3. ISOLATION AND CHARACTERIZATION OF HALOPHILIC BACTERIA AND ARCHAEA FROM SALT PONDS

Twenty-six isolates were obtained from the solar salt ponds at salinities of 100, 150, 200 and 250 in Hangu Saltworks through agar plate culture. Phylogenetic analysis of 16S rRNA gene sequence indicated that they were related to five bacteria genus *Halomonas*, *Salinicoccus*, *Oceanobacillus*, *Gracibacillus* and *Salimicrobium*, and one archaea genera *Halorubrum* (Deng et al., 2014).

It had been commonly assumed that the microbial biodiversity in moderate environments are mostly contributed by halophilic bacteria, while archaea are predominantly existed in crystallizers (Olsen, 1994). Nevertheless the recently published molecular data have indicated the presence of archaea in moderate environments, while bacteria seem to be widespread as archaea are (Antón et al., 2000; Oren, 2002). In our study, only five bacterial genus were found at salinity of less than 200 and one archaeal genus were observed in crystallizer pond at salinity 250. *Halomonas* sp. was found to be predominant and wide-spread genus among the isolates, accounting for 30% of the total isolates. Moreover six colonies recovered from brine at salinity 250 were all identified as *Halorubrum* sp.

Based on the genus and the original sampling salinity, eight bacterial isolates and two archaeal isolates were selected for further morphological, physiological and biochemical characterization. All the bacterial strains were found to be moderately halophilic with the optimal growth salinity 50 or 100, whilst two archaeal strains were extremely halophilic with the optimal growth salinity 200 (Deng et al., 2014).

4. BIOACTIVE COMPOUND ACCUMULATION IN HALOPHILIC BACTERIA AND ARCHAEA

Poly- β -hydroxybutyrate (PHB) is a lipid-soluble compound reserved in cells of prokaryotes. It has unique characteristics of being biodegradable, biocompatible and thermoplastic microbial polyester thus has a great potential of commercial application in the field of environmental protection, medicine (Lauzier et al., 1994) and aquaculture (Defoirdt et al., 2011). We have investigated the effect of increasing glucose supplementation on growth and PHB content of *Halomonas* sp. using basal culture medium containing 7.5 g/L acid-hydrolyzed casein. The results showed that glucose supplementation in the culture medium significantly improved the growth and PHB content of *Halomonas* sp. At a glucose concentration of 30 g/L, the cell dry weight and PHB content reached 8.60 g/L and 5.57 g/L (65 % cell dry weight) (Sui et al., 2014).

The red coloration of brine is mostly due to the abundant presence of C₅₀ bacterioruberin and its derivatives in cell membranes of archaea (Oren, 2002). A number of biological functions, such as improving rigidity and fluidity of the cell membrane, protecting the cells against oxidation, strong light injury, and DNA damage have been reported (Litchfield, 2011). Bacterioruberin production through halophilic archaeal cultivation and its potential application have recently aroused interests from many scientists. We have studied the effects of salinity and pH on accumulation and composition of the pigments of two archaeal strains, namely *Halobacterium* Strain SP-2 and *Halorubrum* Strain SP-4. Their optimum salinity and pH for growth were 25% and 7, respectively. The pigment accumulation (OD490/mL broth) was the highest at pH 8. In addition, the data recorded in salinity range of 15%-30% revealed that increasing salinity conducted to a decreasing rate of pigment accumulation. The analysis of UV-Vis spectrum, TLC and HPLC chromatograms showed that C₅₀ carotenoid bacterioruberin is the major pigment in both strains (Sui et al., 2014).

5. CONCLUSIONS

Solar salt ponds in Bohai Bay Saltworks inhibit diverse microbial flora verifying with season and brine water salinities. A number of halophilic bacteria and archaea strains were isolated and characterized from Bohai bay salt ponds. Studies of the bio-active compounds accumulation such as PHB and C₅₀ carotenoid in specific halophilic bacteria (e.g. *Halomonas* sp.) and archaea (e.g. *Halorubrum* sp.) provided valuable data with respect of utilization of brine water biological resources.

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