



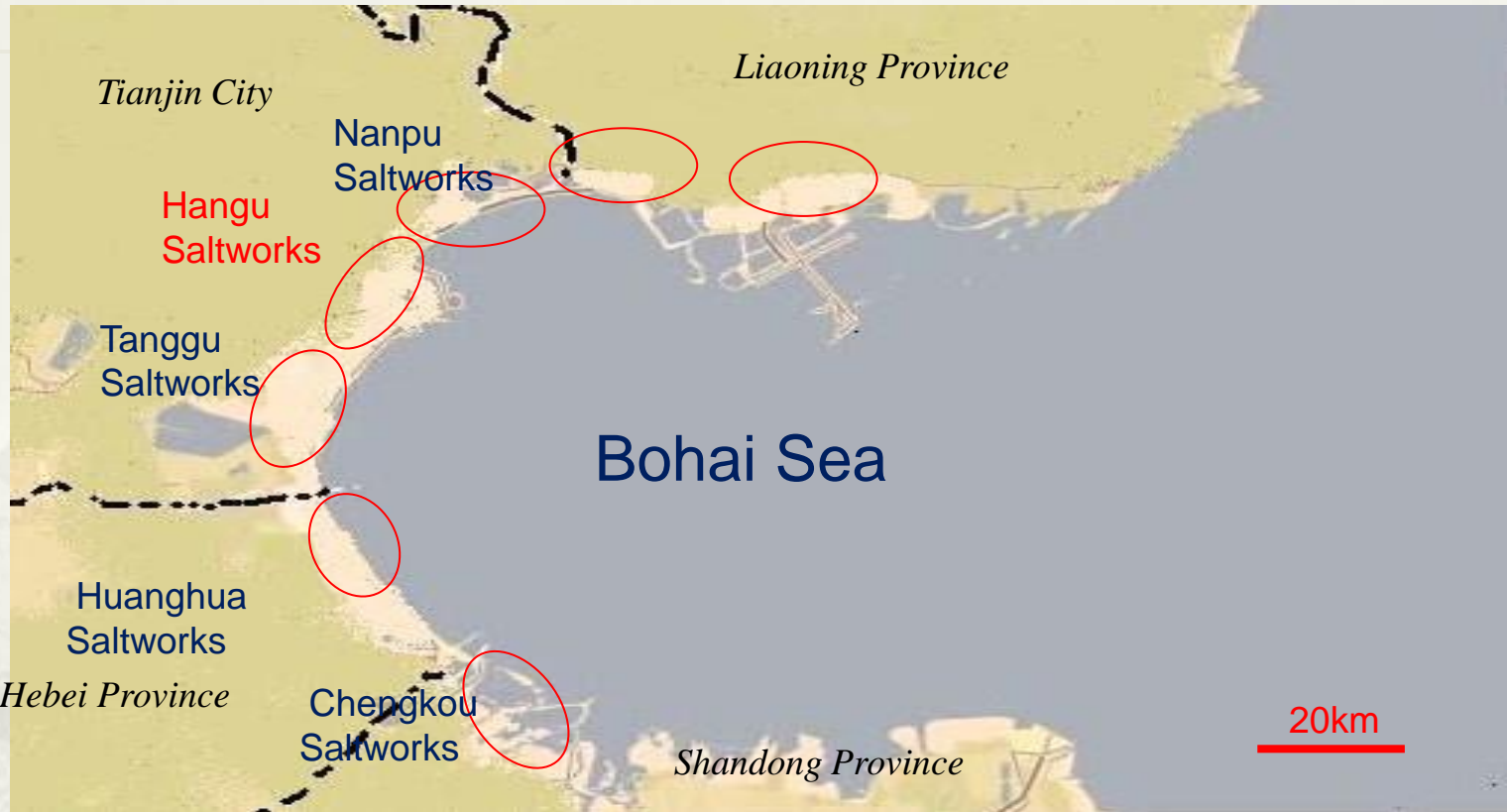
# Microbial biodiversity in Bohai Bay saltworks and their biotechnological utilization

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# Bohai Bay Saltworks



**A major sea salt producing area: covering 1500 km<sup>2</sup>**

**A total annual salt yield: 20 million tons in 2011 (China Salt Association).**

# Aquaculture activities in Bohai Bay saltponds

Salinity

Primary  
evaporation  
(Seawater-12°B é )

Medium-grade  
concentration  
(12-17 °B é )

High-grade  
concentration  
(17- 25°B é )

Crystallizers  
( >25 °B é )



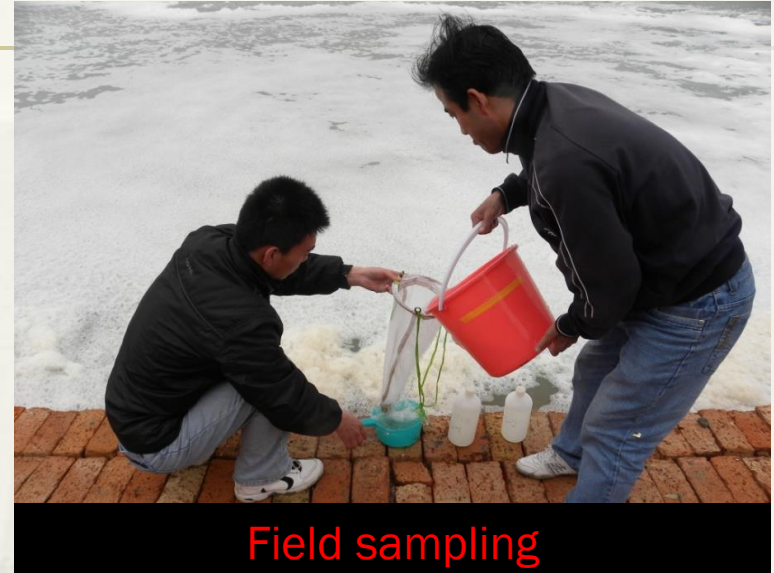
Production of *Artemia*  
biomass and cysts



# Microbiota in solar saltworks

- \* Along the salinity gradient, the majority of microbial community changes from **moderately halophiles** to **extremely halophiles**, and biodiversity decreases as the environment become more hypersaline (Oren, 2002).
- \* In the crystallizers, the blooms of red halophilic bacteria and archaea ensure the increased heat absorption, the reduction of the dissolved organics, eventually resulting in **the enhanced evaporation and improved salt crystallization**.
- \* Some halophilic bacteria and archaea contain abundant active compounds, such as **poly-hydroxyalkanoates (PHA)**, **carotenoid pigments** and **polysaccharides**, which can be utilized in medicine, food industry and environmental protection, etc.

# 1. Bacterial Biodiversity Study



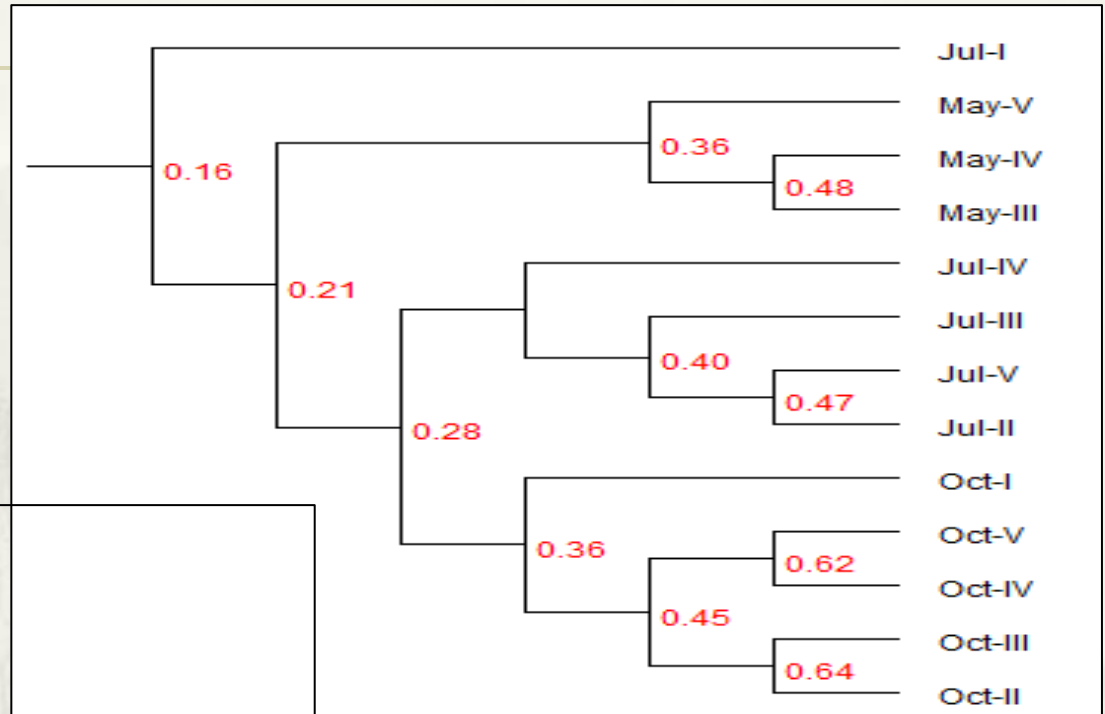
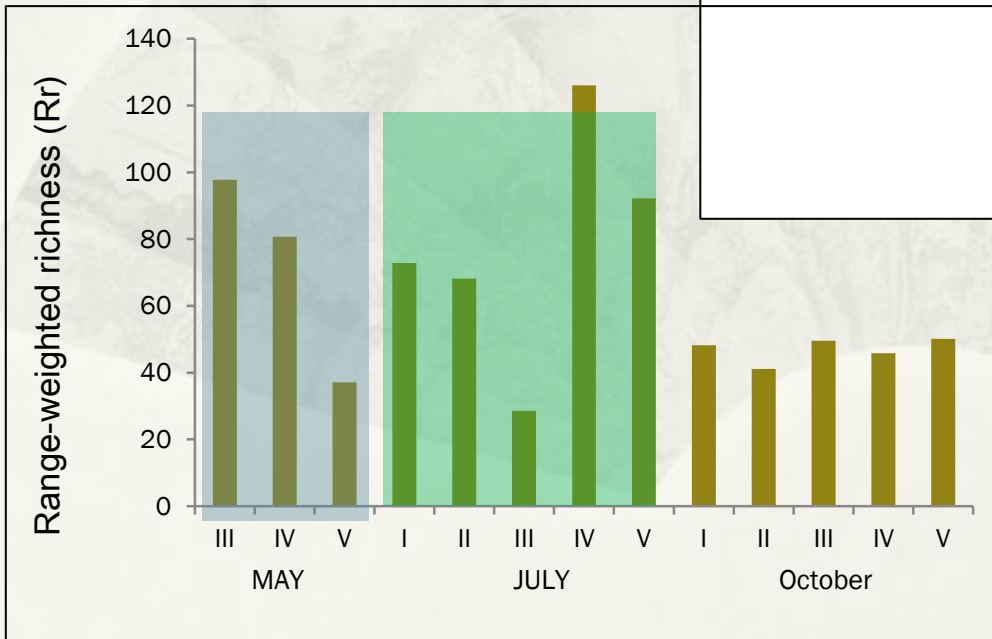
Field sampling

Sampling station	Salinity (°Bé)		Temperature ( °C )		
	Expected	Measured	May	July	October
I	5	4.41-7.29	18.1	28.3	15.3
II	8	7.91-9.24	18.7	27.9	15.1
III	12	10.79-11.34	19.4	28.1	16.3
IV	16	12.34-15.05	20.1	28.2	16.5
V	20	16.36-18.51	22.2	28.4	18.3



# DGGE clustering analysis of brine water in saltponds

- Temperature had more effect on the bacterial community than salinity.

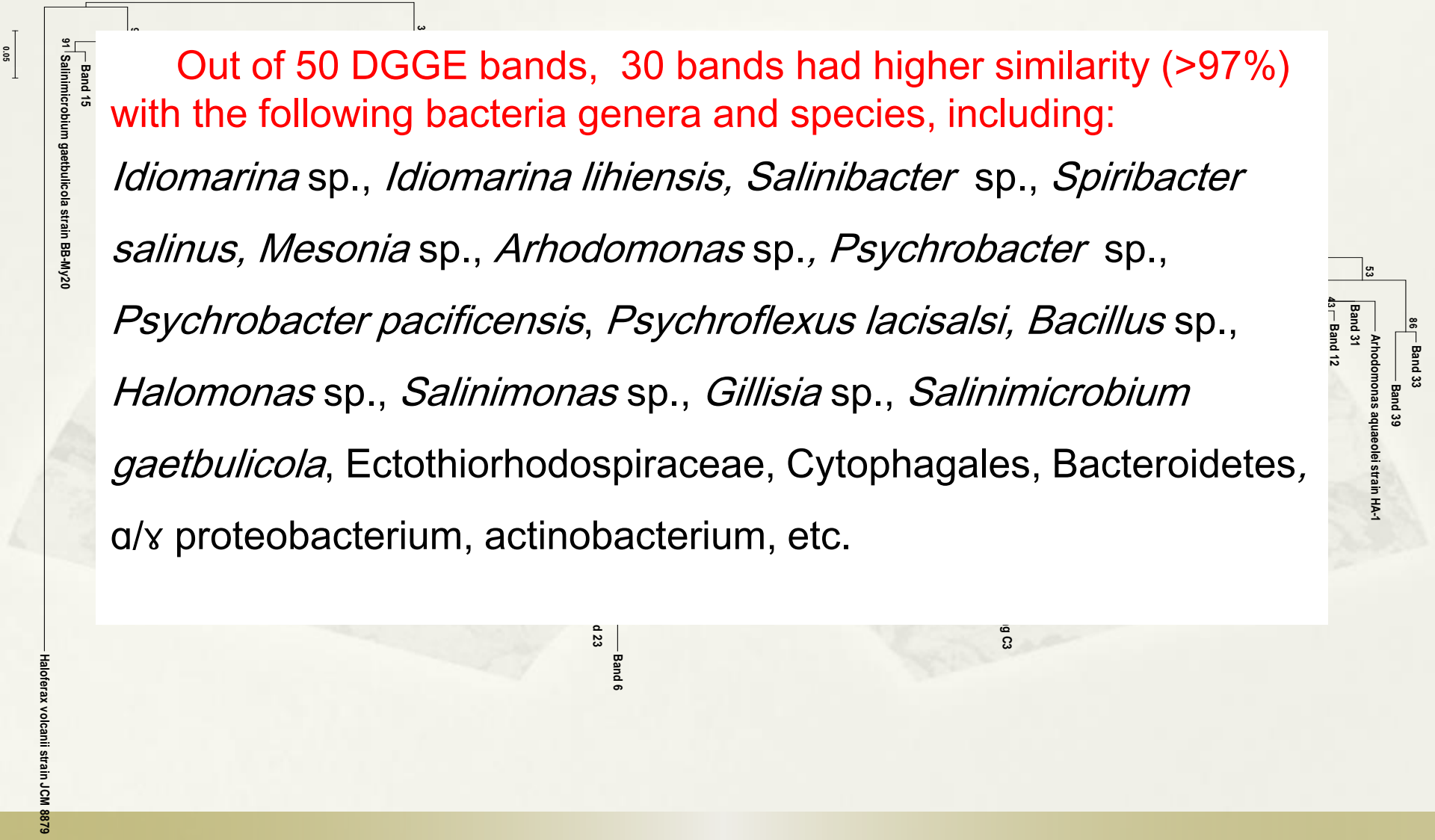


- Biodiversity index (Range-weighted richness)  $R_r > 30$ , indicates that the investigated saltponds have high diversity (Boon et al., 2003).

# Phylogenetic analysis on 16S rDNA recovered from DGGE bands

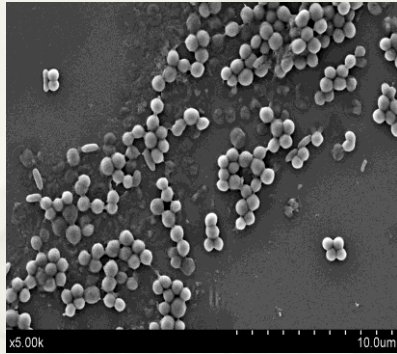
Out of 50 DGGE bands, 30 bands had higher similarity (>97%) with the following bacteria genera and species, including:

*Idiomarina* sp., *Idiomarina lihiensis*, *Salinibacter* sp., *Spiribacter salinus*, *Mesonina* sp., *Arhodomonas* sp., *Psychrobacter* sp., *Psychrobacter pacificensis*, *Psychroflexus lacisalsi*, *Bacillus* sp., *Halomonas* sp., *Salinimonas* sp., *Gillisia* sp., *Salinimicrobium gaetbulicola*, Ectothiorhodospiraceae, Cytophagales, Bacteroidetes,  $\alpha/\gamma$  proteobacterium, actinobacterium, etc.

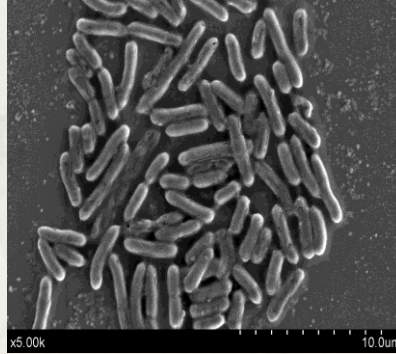


## 2. Isolation and Identification of Bacterial and Archaeal Strains

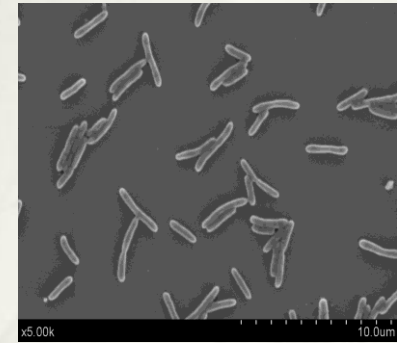
More than 20 strains were identified and characterized from saltponds with salinity of 100, 150, 200 and 250 g/L in Hangu Saltworks.



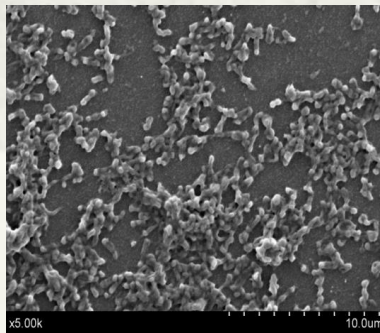
*Salinicoccus* sp.



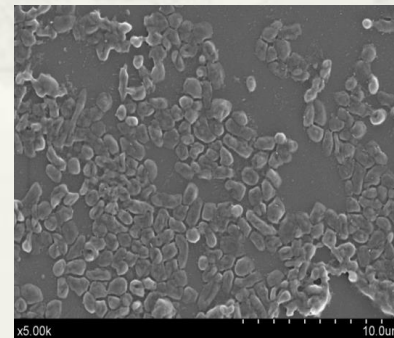
*Halomonas* sp.



*Salimicrobium* sp .



*Halobacterium* sp.

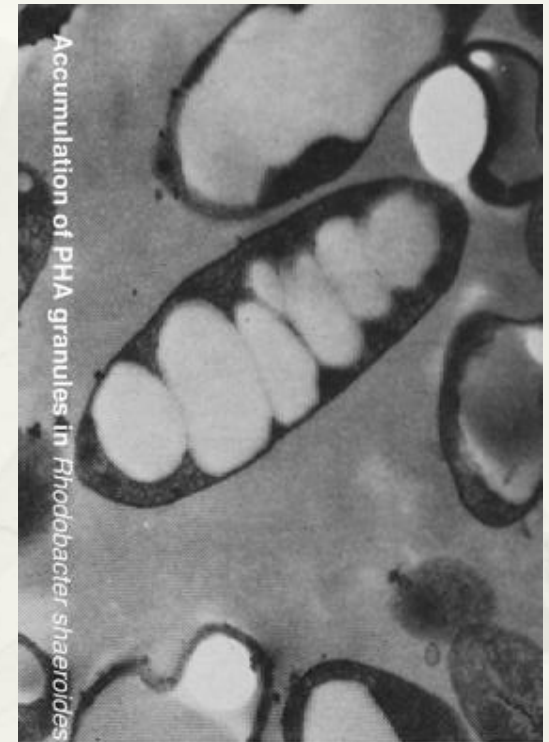


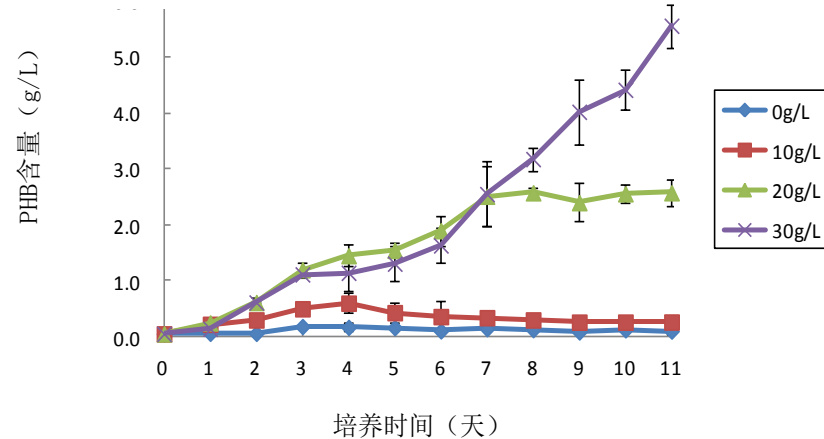
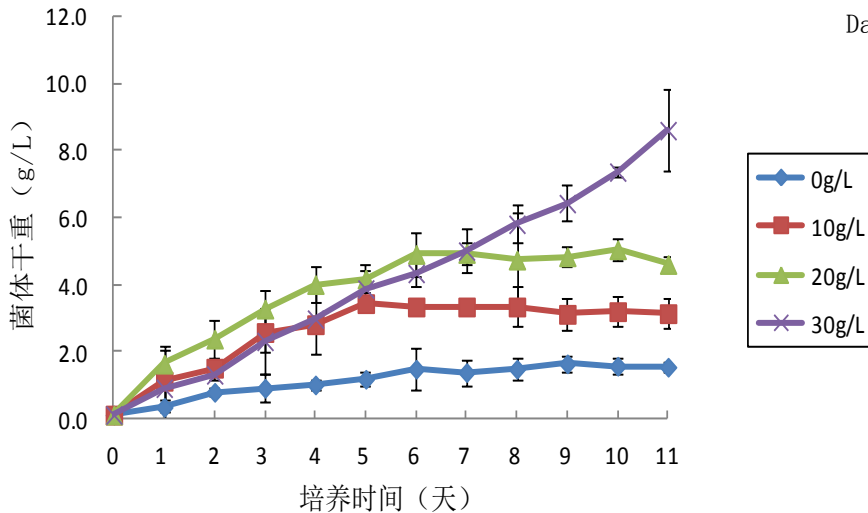
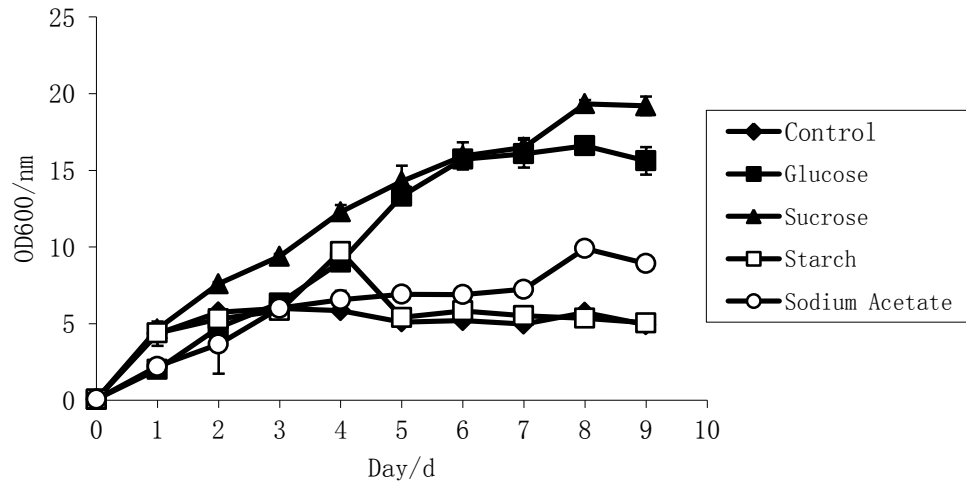
*Halorubrum* sp.



### 3. PHB Accumulation in *Halomonas* sp.

- \* Poly- $\beta$ -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.
- \* Some bacteria and archaea are able to accumulate PHB in condition of excess carbon, e.g. *Halomonas boliviensis* , *Haloferax mediterranei* and *Haloarcula* sp.



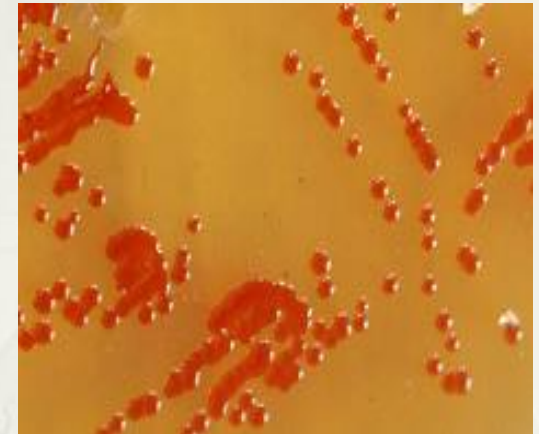


Glucose and sugar are better carbon sources for growth of *Halomonas* sp. Increasing glucose supplementation significantly improved growth and PHB content in *Halomonas* cells.

At a glucose concentration of 30 g/L, the cell dry weight and PHB content reached 8.6 g/L and 5.6 g/L (65 % cell dry weight).

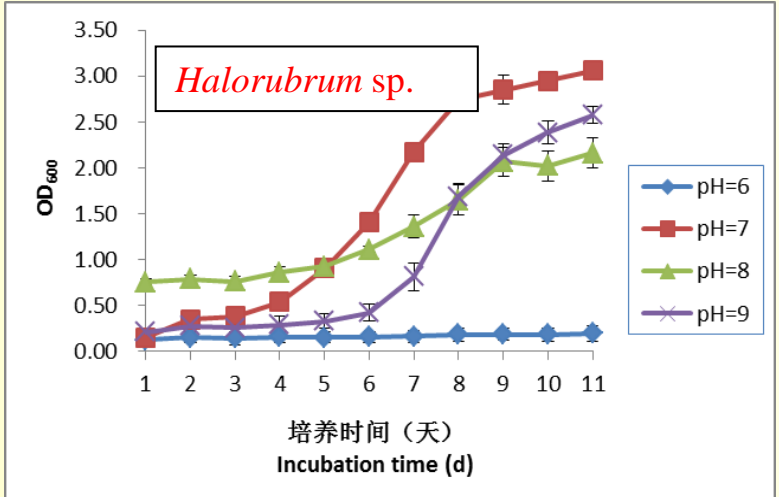
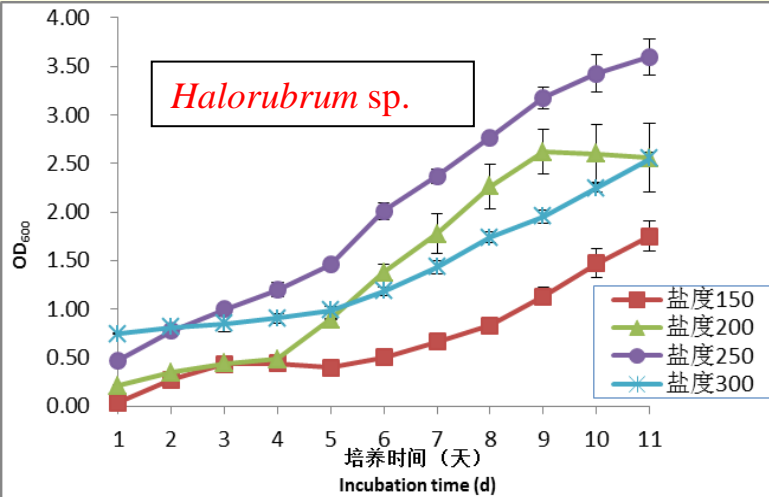
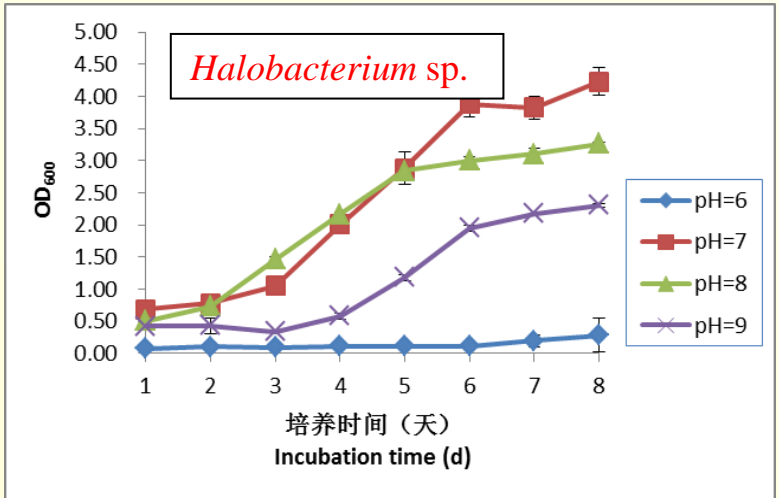
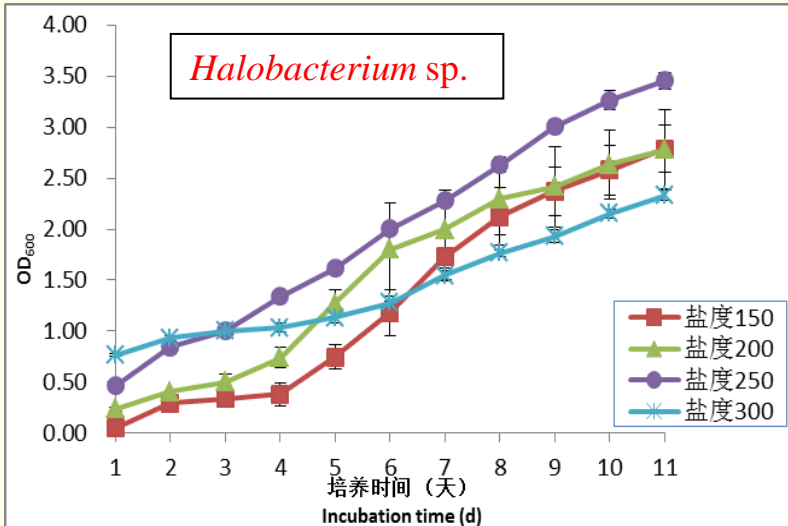
## 4. Bacterioruberin Accumulation in *Halorubrum* and *Halobacteria*

- The red coloration of brine is mainly due to the abundance of C<sub>50</sub> bacterioruberin and its derivatives in the cell membranes of archaea.
- Bacterioruberin has a number of biological functions, such as improving the rigidity of the cell membrane, and protecting cells against oxidation, strong light injury and DNA damage.



Red colony *Halorubrum*

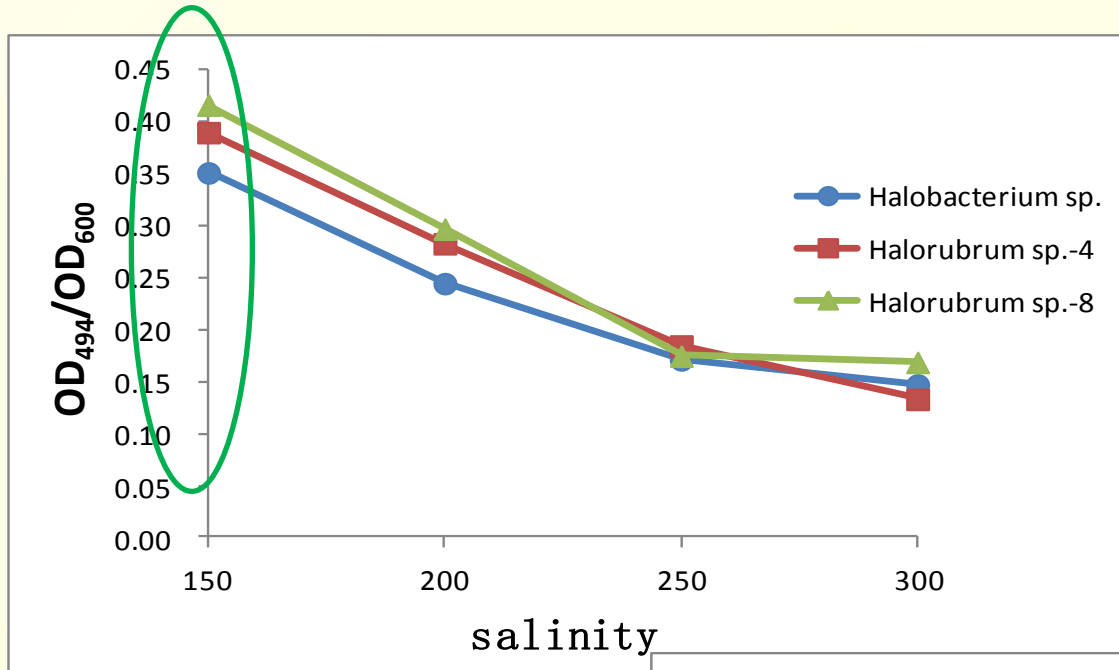
# Optimum salinity/pH on growth of *Halobacteria* and *Halorubrum* sp.



Optimal salinity for growth — 25 °Bé

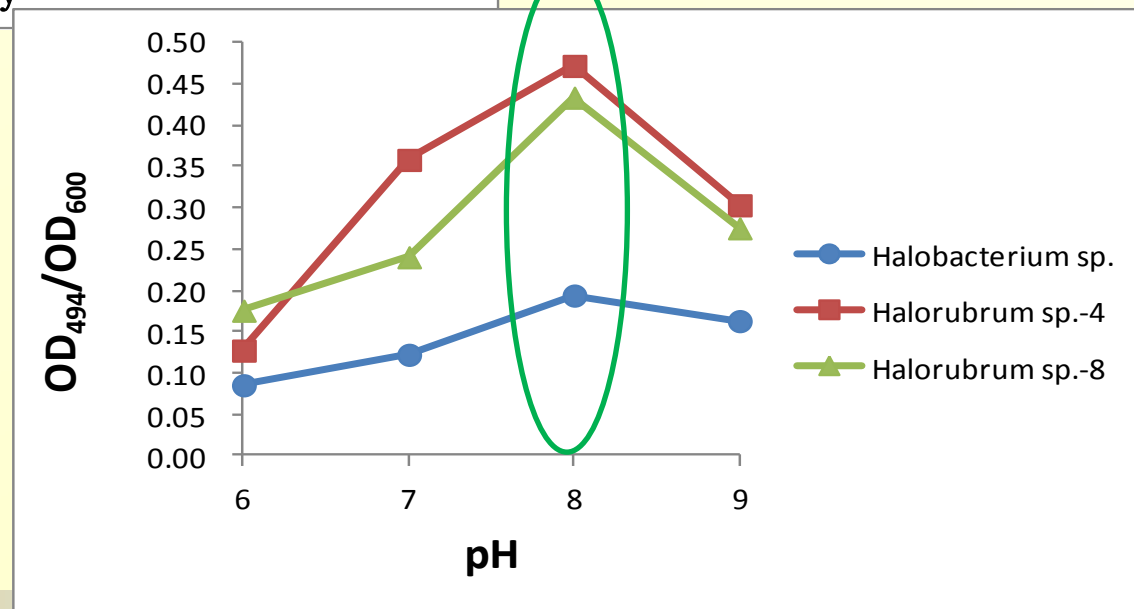
Optimal pH for growth — pH7

# Optimal salinity /pH on pigment accumulation in two strains



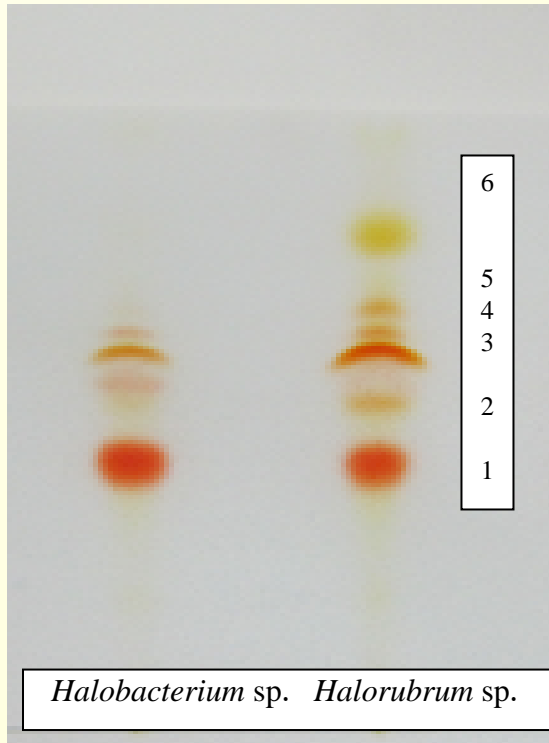
Pigments accumulation was higher at salinity 15 °Bé

Pigments accumulation was higher at pH 8.

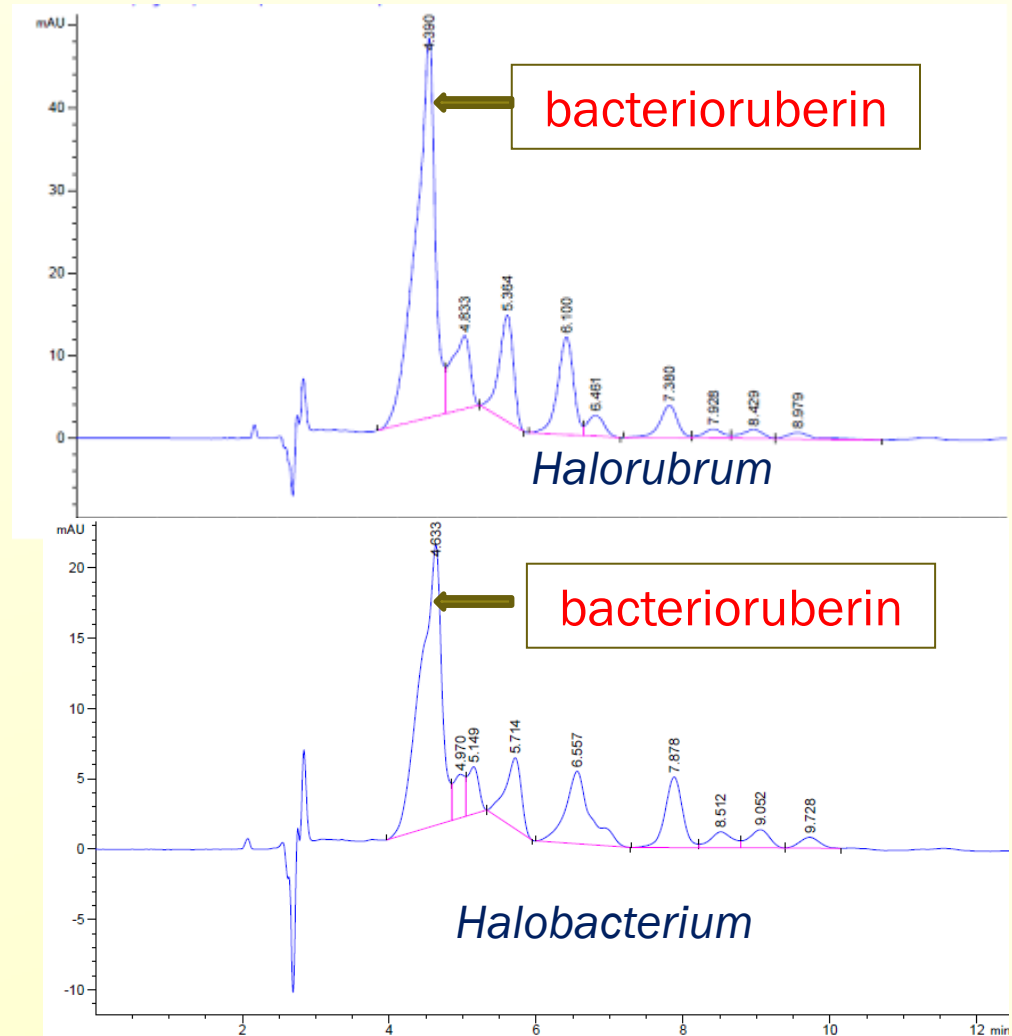




# Pigment composition analysis



Thin-layer chromatogram of pigment extracts from *Halobacterium* and *Halorubrum*



HPLC spectrum of pigment extracts

**Thank you**

