

## ENDOLITHIC HALOPHILES FOUND IN EVAPORITE SALTS ON TIBET PLATEAU AS A POTENTIAL ANALOG FOR MARTIAN LIFE IN SALINE ENVIRONMENT.

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**Introduction:** Da Langtan playa occurs at the west end of Qaidam basin, on northern part of Tibet plateau (or Qinghai-Tibet Plateau) [1]. It is classified as a Hyperarid region on a plateau with the highest average altitude (4500 m) on the earth. Mg-sulfates was found within the evaporative salt deposits of the playa [2], similar as those found on Mars [3, 4, 5, 6, 7, 8, 9]. Recently, widespread chloride-bearing materials were implied in regions of the southern highlands of Mars [10]. The geomorphology of these deposits is consistent with formation in evaporate environments. Study of endolithic halophiles can provide clues for the understanding of life strategies in the extreme terrestrial environments, which can serve as an analog for the potential Martian life in saline environment. In the past, some microorganisms from the evaporative crusts of arid region were found and investigated. They showed that halite rocks are colonized by *Chroococcidiopsis* and associated heterotrophic bacteria in the driest parts of the Atacama Desert [11]. In this abstract, we report the halophiles isolated from the evaporative salts in a hyperarid and hypersaline environment on Tibet Plateau for analogs of the search for Martian life in subsurface.



Fig.1 Sample sites at Dalangtan playa, A. evaporite crust; B. salt crystal in salt lake; C. deposits of cross sections. Arrows showed sample collection positions

**Sample sites description:** The studied samples are evaporate crusts, salts crystals, and the samples in cross sections (Fig. 1, A, B, C) collected at the Da Langtan playa during the Oct. 2008 field expedition. In this area, average temperature is  $-2^{\circ}\text{C}$  and annual precipitation is less than 25 mm [13]. Evaporative salty crusts cover the entire playa without any vegetation at the surface. The major salts within crusts are halite and

gypsum. Mg-sulfates with different hydration degrees, including kieserite, starkeyite, and pentahydrate were found in the top layer of deposition sequence. Ca- and Mg-carbonates were found in the surface soil, and also coexisting with subsurface sulfate deposits [2].

**Isolation and pure culture of Halophiles:** The samples *DLT-10*, *DL-S-2*, and *DP-3* from evaporative crust, salt crystals, and deep deposits were used to isolate the microbes in laboratory. The extracted halophiles were cultured on the modified growth mediums (MGM) with different salinities of 12%, 18%, 23%, 25% respectively.

**Microscope observation of halophiles:** After 2 days of cultures, only one dish of 12% MGM had colonies grown from sample *DL-S-2*. Two weeks later, ten colonies grew from *DLT-10*, *DL-S-2*, and *DP-3* on 12% MGM, and two colonies on 18% MGM. The shape of colonies was white, round with diameter 2-3 mm (Fig. 2A, B). All bacteria showed Gram negative. The shapes of bacteria under microscope are rod or coccoid, with size of 2-3  $\mu\text{m}$  (Fig 2 C, D). Until the time of this report, we have not found any colony grow on 23% MGM and 25% MGM.

**Phylogenetic tree of the halophiles:** Genomic DNA was extracted from the cultured halophiles, and 16S rRNA gene were amplified using the primers: 530F: GTGCCAGCMGCCGCGGTAA, 1492R: TACGGYTACCTTGTTACGACTT. The amplification was carried out in Biorad *iCycler* DNA Thermal Cycler with the following amplification program:  $94^{\circ}\text{C}$  for 5 min; 30 cycles of  $94^{\circ}\text{C}$  for 30 sec,  $55^{\circ}\text{C}$  for 30 sec,  $72^{\circ}\text{C}$  for 30 sec, and  $72^{\circ}\text{C}$  for 10 min extension. The amplified products were fractionated in 1.2 % agarose gel and detected with UV photography system. 1 kb bands were successfully amplified from 9 strains of halophiles (Fig.3). Afterwards, the PCR products were ligated to pGEM-T vector and sequencing was carried out. 16S rRNA gene sequences for 8 strains of halobacteria were achieved.

Homologous analyses for the sequences of 8 strains of halobacteria (*DLT-10-1*, *DLT-10-2-1*, *DLT-10-2-3*, *DLT-10-2-6*, *DP-3-3*, *DL-S-2-4*, *DLT-10-5*, and *DL-S-2-6*) were carried out by BLAST search in database of GenBank [12]. The phylogenetic tree was constructed using software DNAMAN (Fig. 4). The results showed that the halophiles found at Da Langtan

had high homology with some species of genera of *Virgibacillus*, *Oceanobacillus*, *Halobacillus*, and *Terribacillus*.

**Conclusion and future work:** We have successfully extracted and cultured a group of halophiles from a hyperarid and hypersaline environment in Qaidam basin, on Tibet Plateau. The extracted halophiles only grew on 12% and 18% MGM, so they belong to moderately halophilic bacteria. Because the extremely halophilic bacteria are generally anaerobic, there may be such kinds halophiles at Da Langtan but was failed to be recovered this time from the evaporative crusts. The culture independent technology should be used to analysis the diversity of halophiles and the physiology of these halophiles will be studied next step. This study would be important to reveal the surviving mechanism of halophiles in an extremely hash environment. The climatic conditions of Da Langtan playa bear certain similarity with those on Mars, so the study of halophiles from Da Langtan may serve as a potent analog for the search of Martian life.

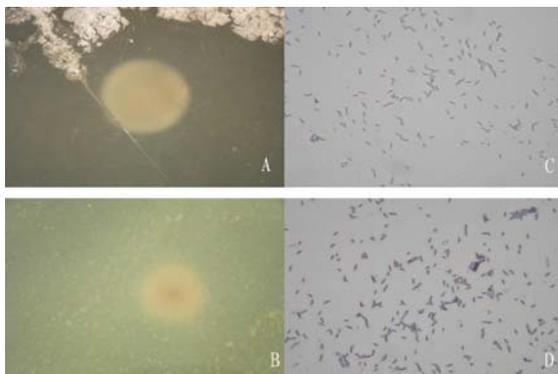


Fig2. The morphology of colonies and microscope shape of halophiles. A. DL-S-2-4; B. DLT-10-2-1; C. DL-S-2-4, bacteria average size is 2.7  $\mu$  m; D. DLT-10-5, bacteria average size is 3.3  $\mu$  m.

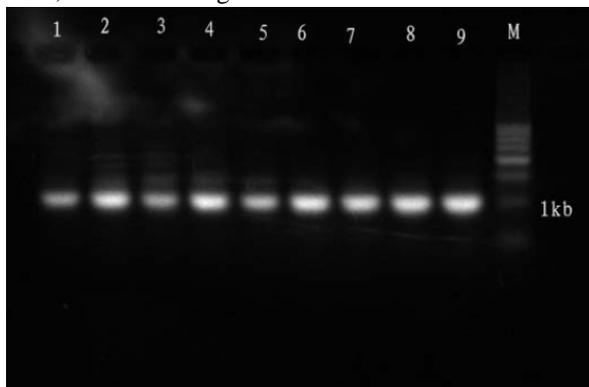


Fig3. PCR products were checked on 1.2% agarose gel. The size was 1kb with primer 530F and 1492R, which designed according the consensus sequence of *E.coli*

16S rRNA gene .Land M. 100bp ladder marker.Land 1-9: 1. DLT-10-1; 2. DLT-10-2-1; 3. DLT-10-2-2; 4. DLT-10-2-3; 5. DLT-10-2-6; 6. DP-3-3; 7. DL-S-2-4; 8.DLT-10-5; 9.DL-S-2-6

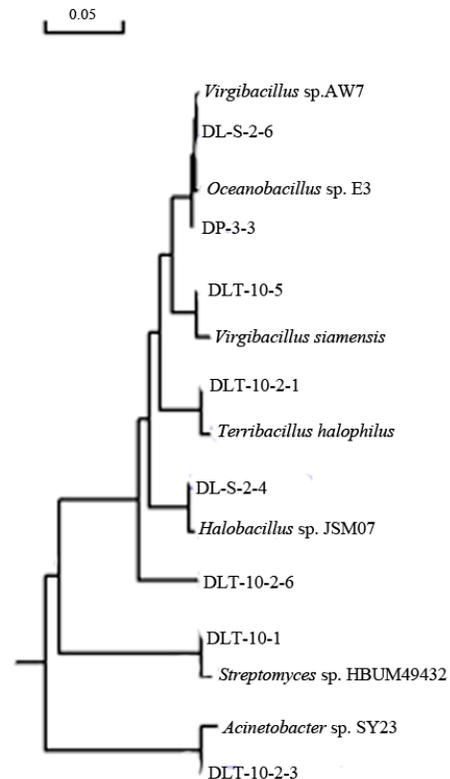


Fig4. Phylogenetic relationships of halophiles isolated in this reports with the homology bacteria BLAST search in GenBank based on the 16S rRNA gene partial sequences.

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