MICROBES IN EVAPORITE SALTS ON TIBET PLATEAU AS ANALOG FOR MARTIAN LIFE IN SALT ENVIRONMENTS. F. J. Kong, M. P. Zheng[1], A. L. Wang[2], N. N. Ma[1]. Jan Amend[2] [1] R&D Center of Saline Lakes and Epithermal Deposits, Institute of Mineral Resources, Chinese Academy of Geological Sciences, Beijing,100037, China (kfjbj2002@yahoo.com.cn), [2]Dept. Earth and Planetary Sciences and McDonnell Center for Space Sciences, Washington University, St. Louis, MO, 63130 USA (alianw@levee.wustl.edu).

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 hyper arid 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.

Materials and Methods: *Sample sites description* The studied samples are evaporate crusts, salts crystals, and the samples in cross sections collected at the Da Langtan playa during the Oct. 2008 field expedition[14]. In this area, average temperature is -2℃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, starkeiyite, and pentahydrite 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 microbes were cultured on the modified growth mediums (MGM) with different salinities of 12%, 18%, 23%, and 25% respectively.

*Microscope observation of microbes***:** Gram staining was carried out. The Cells were observed under light microscope, and the photos were taken by digital camera.

*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 Bio-Rad *iCycler* DNA Thermal Cycler with the following amplification program: 94℃ for 5 min; 30 cycles of 94℃ for 30 sec, 55℃ for 30 sec, 72℃ for 30 sec, and 72℃ for10 min extension. The amplified products were fractionated in 1.2 % agarose gel and detected with UV photography system. 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.

The growth of halophiles in different salinity : The strains of halophiles were cultured in liquid media contained 5%, 12%,18%,23%,30% salt at 37 ℃ for 24h.The absorption value of cultures was measured at wave length 660 nm.

Nano scanning microscopy: The samples and bacteria were prepared on the special plate and the image was observed using the instrument FEI NOVA NanoSEM 2300. The element composition of sampletarget area were measured by EDAX.

Results: After 2 days of cultures, only one dish of 12% MGM had colonies grown from sample DL-S-2. Two weeks late, ten colonies grew from *DLT-10*, *DL-S-2*, and *DP-3* on 12%MGM, and two colonies on 18% MGM. We have not found any colony grow on 23% MGM and 25% MGM. The shape of colonies was white, round with diameter 2-3 mm. All bacteria showed Gram negative. The shapes of bacteria are rod or coccoid, with size of 1-3μm (Fig 1).

16S rRNA gene sequences for 8 strains of halobacteria showed that the halophiles found at Da Langtan had high homology with some species of genera of *Virgibacillus*, *Oceanobacillus*, *Halobacillus*, and *Terribacillus.*

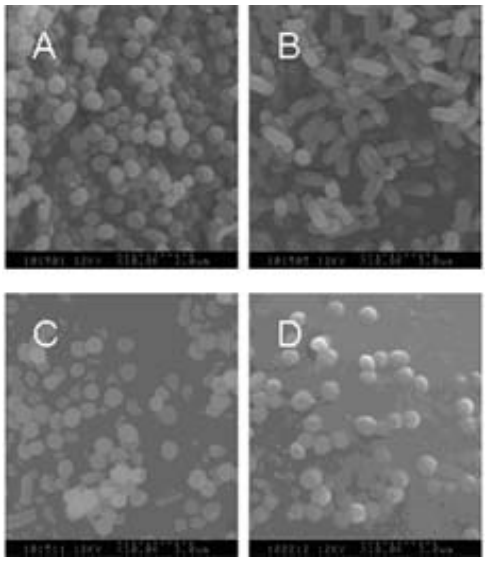

Fig1. The SEM micrographs of the halophiles. A.2-1; B.2-2-1; C.4; D.2-6

8 strains showed different growth curve in different salinity (Fig 2). The opium salinity for growth were 5%(strain 2-1, 2-3-1, 2-3-2, 3), 18%(strain 5), and without salt(strain 2-2-2,2-6). The salinity scale of growth for strain 5 was 5-30%, 0-12% for Strain 2-6, 0-18% for strain 2-2-2,2-3-2, 3, 0-23% for strain 2-1, 0-30% for strain 2-1.So, the bacteria isolated were moderate halophiles

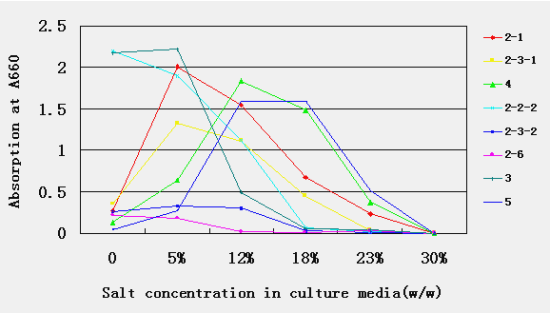

Fig 2. The growth curve of strains 2-1(DLT-10-2-1),2-3-1(DLT-10-2-3-1),4(DLS-2-4), 2-2-2(DLT-2-2-2),2-3-2(DLT-10-2-3-2),2-4(DLT-10-2-4),3(DP-3-3),5(DLT-10-5) in different salinity culture media.

The SEM of halophile and EDAX were carried out to find the microbial biosignatures in the evaporate crystal sample. The results showed the active and fossil microbial signature could be found. (Fig3, Fig 4).

Future work: The survival mechanism for halophiles in hyperarid and hypersaline environment on Tibet Plateau would be studied in the future.

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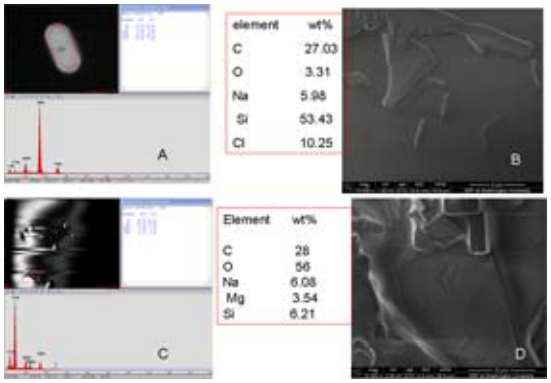

Fig3. The SEM micrographs of the halophile and its element composition by EDAX.

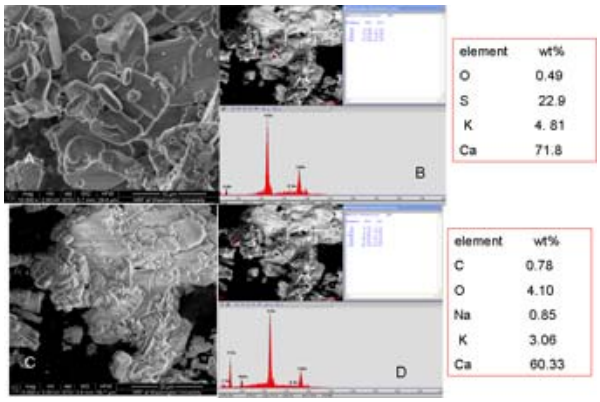

Fig 4.The SEM image of evaporate crystal sample DLT-10 and the element composition of microbial like morphology by EDAX.

References: References: [1] Zheng et al. (2009) 40th LPSC. #1454. [2] Wang et al (2009), 40th LPSC. #1858. [3] Clark et al. (2005), *EPSL, 240*, 73-94. [4]Wang, A. et al. (2006), *JGR, 111*, JE002513, JE002516. [5] Squyres et al. (2006), *JGR, 111*, JE002771. [6] Bibring et al. (2005), *Science*, V307, 1576-1580. [7]. Arvidson et al. (2005) *Science 307*, 1591-1593. [8] Gendrin et al. (2005), *Science 307*, 1587-1591. [9] Langevin et al. (2005), *Science, 307*, 1584-1586. [10] Osterloo M. M., et al. (2008) *Science, 139*,1651-1654. [11] Wierzchos, J. et al. (2006) *Astrobiology*,3,415-422.[12]http:// *blast.ncbi.nlm.nih.gov*. [13] Sun Hong lie et al. (1990), *Atlas of the Qinghai-Tibet Plateau*, Beijing: Science Press, 69[14] Kong et al. (2009) 40th LPSC. #1216.