

**MICROBIAL ANALYSIS OF DALANGTAN PLAYA: A MARS ANALOGUE AND ITS ASTROBIOLOGICAL SIGNIFICANCE.** T. Huang<sup>1</sup>, L. Xiao<sup>1</sup>, H. Wang<sup>2</sup>, C. Escudero<sup>3</sup>, J. M. Martínez<sup>3</sup>, R. Amils<sup>3</sup>, and R. Wang<sup>2</sup>. <sup>1</sup>Planetary Science Institute, China University of Geosciences, Wuhan 430074, China. huanglotte@cug.edu.cn, <sup>2</sup>State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Wuhan 430074, China. <sup>3</sup>Centro de Biología Molecular “Severo Ochoa” (UAM-CSIC), Madrid 28049, Spain.

**Introduction:** Dalangtan Playa is located in northwest of Qaidam Basin (NW, China) with hypersaline and arid condition. Due to the average high altitude (over 2700 m), the UV radiation is very high (e.g. the monthly surface downwelling shortwave radiation is 266.6 KJ/m<sup>2</sup>·S). This area has been proposed as a perfect analogue for Mars evaporitic condition with astrobiological significance [1, 2]. Previous mineralogical studies as well as in-situ monitoring indicated the existence of mineralogical processes similar to those described on Mars [3, 4]. The presence of preserved subsurface microbial lipids in recent 123.5±13 ka has also been investigated [5]. However, little has been done on microbial diversity and abundance in this region. To better understand the habitability in this extreme environment, both surface and subsurface samples were collected for further mineralogical and microbial analysis (Fig 1).

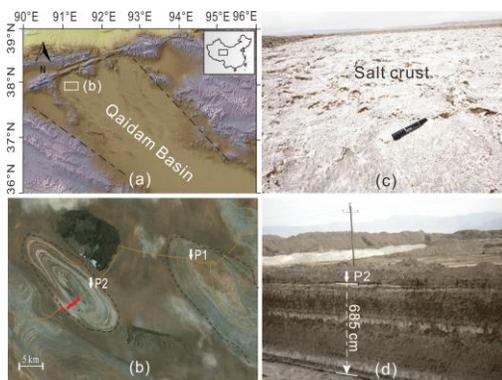


Fig 1. Studying site and sampling locations. (a) Relief shading map of the Qaidam Basin based DEM (ASTER GDEM). Sampling locations are shown in (b), in which white arrows indicate locations for subsurface sampling profile P1 and P2, red points are for 7 surface sampling sites. (c) Salt crusts on the surface of Dalangtan Playa. (d) Profile of P2.

**Analytic Methods:** X-ray diffraction analysis (D8-FOCUS, BRUKER Ltd.) of sediments were done for mineral assemblage. Quantitative determination of total organic carbon of samples were analyzed with vario Macro cube (Elementar, Germany). Moisture contents of samples were measured in the State Key Laboratory

of Biogeology and Environmental Geology (Wuhan, China). Both culture-independent and -dependent methods were used for microbial identification. DNA extraction methods were used for microbial diversity identification. CAtalyzed Reporter Depositon-Fluorescence In Situ Hibridization (CARD-FISH) was used to detect and quantify cell numbers. Isolation techniques with R2A, MGM and AM media supplemented with NaCl in different concentrations were used to isolate microbes from sediment samples.

**Results:** The mineral composition of the surface samples were mainly chlorides and gypsum. While for the subsurface samples, they were composed by evaporates and silicate mineral fragments which revealed one or more sequences of lacustrine sedimentation. Total organic carbon was generally low. The lowest value was 0.03% for a surface sample dominated by halite (~50%) and thenardite (~45%). Moisture content of the samples was as low as 0.08%.

Twelve selected environmental samples were subjected to DNA extraction. The concentration of DNA products of the samples was between 1.0 to 5.0 ng/μl. Amplification using archaeal (Arc 1F, 1R), bacterial [(21F, 1492R) and (S-D-Bact-0341-b-S-17, S-D-Bact-0785-a-A-21)] and eukaryotic (563F, 1132R) primers did not give positive results as indicated by gel electrophoresis of PCR products. No inhibitors for amplification were detected.

CARD-FISH and DNA stain were applied for the identification and quantification of the indigenous microbial community in the original sediments. Bacterial clusters were efficiently hybridized by CARD-FISH with EUB338 I-III mix probes (Fig 2). A total of  $\sim 2 \times 10^5$  bacteria g<sup>-1</sup> in average was counted for subsurface samples of P1 and  $\sim 3 \times 10^5$  bacteria g<sup>-1</sup> for those of P2. Hybridization with Firmicutes specific probe (LGC354a, b) indicated that 77% and 76% of bacteria for P1 and P2 belong to this phylum respectively. No signals of archaea were detected with probe ARC915.

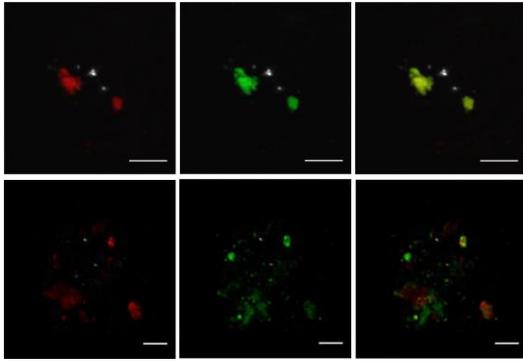


Fig 2. Fluorescent microscopy analysis of subsurface samples from profile of P2 of Dalangtan Playa. (a) 355 cm deep, and (b) 285 cm deep. From left to right: epifluorescence micrographs showing bacterial cells hybridized with the universal probe EUB388I- III mix, the same field stained with Syto9, and the overlap of stained field. Scale bars: 5  $\mu$ m.

A total of 54 aerobic heterotrophic bacteria and 7 fungi were isolated from 22 out of 23 samples along the two profiles and 3 of 7 surface samples. No archaea has been isolated up to date.

Bacterial isolates showed  $\geq 97\%$  16S rRNA gene sequence homology with members of *Bacillus* (major species, 24 strains), *Oceanobacillus*, *Halobacillus*, *Gracilibacillus*, *Microbacterium*, *Thalassobacillus*, *Sediminibacillus*, *Ornithinibacillus*, *Pontibacillus marinus* and *Nocardiopsis* genera, which are affiliated with Bacillales (Firmicutes phylum), Actinomycetales and Streptosporangiales (Actinobacteria phylum) orders. Fungal isolates showed  $\geq 98\%$  ITS1 sequence homology with members of *Aspergillus*, *Cladosporium* and *Penicillium* genera affiliated to the Eurotiales and Capnodiales orders of the phylum of Ascomycota.

#### Discussion:

Mineral assemblages of subsurface samples here recorded water history of Dalangtan Playa, which was covered by a lake during Jurassic to late Neogene [6]. Similar evaporates were found on Mars, e.g. sulfate and phyllosilicate have been detected in Columbus crater which is also suggested to have a lake history [7].

In the case of microbial diversity, Dalangtan shows a relatively low microbial biomass similarly to the well-studied Mars analogue Atacama Desert [8]. Firmicutes dominated bacterial isolates, which also corresponded to the results of CARD-FISH technique. This indicates the strong capability of Firmicutes to survive under extreme conditions. This in turn makes this phylum (e.g. *Bacillus* genus) a target for planetary protection measures. Up to date, we have not succeeded ex-

tracting amplifiable DNA from Dalangtan samples probably consequence of the low numbers of microorganisms detected. In addition, considering the existing extreme conditions of aridity and UV radiation in this area, preserved DNA materials in the sediments is expected to be very low. More research must be done to overcome this challenge.

*Plans for future work.* The deep subsurface of Dalangtan Playa is the next target to investigate microorganisms living in the dark and anaerobic condition. Furthermore, brine trapped in pristine salts' crystals should be studied since similar condition on Mars has been suggested to be a good candidate for possible habitat for microorganisms [9]. The last but not the least, the relationship between evaporites and associated microbes in Dalangtan Playa has not been clarified yet, thus more research needs to be conducted.

In total, Dalangtan Playa is a perfect condition for Mars ancient lake analogue. The research in this ecosystem is very limited to date on microbiology as well as on astrobiology. Our study showed a low-biomass but a diverse habitat in this region which strongly calls for further investigation on astrobiology and field planetary testing for life searching missions on Mars.

#### References:

- [1] Anglés A. and Li Y. (2017) IJA, 16, 355-9. [2] Xiao L. et al. (2017) ESR, 164, 84-101. [3] Wang A. et al. (2013) Ica., 226, 980-91. [4] Kong W. et al. (2014) AM 99, 283-90. [5] Cheng Z. et al. (2017). Ast. 17,1112-22. [6] Shen Z. (1993) CLP. [7] Ehlmann B. L. and Edwards C. S. (2014) AREPS. 42, 291-315. [8] Robinson C. K. et al. (2015) EM. 17, 299-315. [9] Gilichinsky D. et al. (2003) Ast. 3, 331-41.