SALT AND OXIDATIVE STRESS ANALYSIS OF EXTREME ORGANISMS IN CRYPTOBIOTIC CRUST TO BETTER UNDERSTAND MARS RELEVANT ADAPTATION STRATEGIES. Dulai S.[1,2], Radnai Zs.[1], Pócs T.[1,2], Pócs T.né[1,2], Tarnai R.[1], Marschall M.[1,2], Kereszturi A.[2,3,4] [1]Eszterházy Károly College, Eger; [2]New Europe School for Theoretical Biology and Ecology, [3]MTA CSFK, [4]NAI TDE (E-mail: ds@ektf.hu)

Introduction: Analyzing the behavior of extreme organisms in cryptobiotic crust for Mars relevant survival [1], after the Chott el Jerid Mars Analog Expedition [2], a new field work was realized in between 14. May – 6. June, 2014 in Turkey (travelling 2800 km, 49 stops, 2.4 kg samples, Fig. 1.) for new samples.

Figure 1. Stops of the expedition in Turkey

Methods: During the simulations crust samples' (from Tunisia and Turkey) photosynthetic activity was analyzed by imaging PAM M-series (Fig. 2.). Different concentration of salts and oxidizers were used (hydrogen peroxide H_2O_2, perchloricacid $HClO_4$, methyl-viologen $C_{12}H_{14}C_{l2}N_2xH_2O$) with 24h rehydration, exposure to actinic light (11, 31, 231, 440 $\mu Em^{-2}s^{-1}$) with stepwise increased concentration until saturation, wash by pure water for recovery (24, 48h) [3]. The lowest light level of 11 µE was equal to Mars like solar radiation under 1-2 mm dust/sand [4], the perchloric-acid's concentration was close to those at the Phoenix's landing site [5], and not from Na/Mg-perchlorate (present on Mars as 0.6 wt % [6]), but more aggressive $HClO_4$.

Results: During the expedition less samples were collected than in Tunisia, and crusts wereg thin and fragile. The best samples were found at Central-Anatolia, the driest region. In a salty lake there, extremely salt *tolerant Flagellatae* type alga was found.

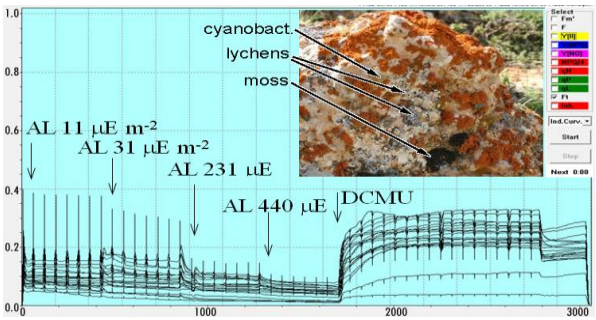

Figure 2. Fluorescence emission traces for quenching analises of intact CBC crusts at different (11, 31, 231, 440 µE m^{-2} s^{-1} actitc light (AL) intensities. The measurements terminate with addititon of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU).

Altogether >600 tests with different light intensity were realized with: NaCl: 43, methyl-viologen: 10, hydrogen peroxide: 10, perchloricacid: 10. Example curves can be seen in Fig. 2. All samples showed certain resistance, the best ones: 13157, 13145E, 13159, 13160D, 13161A. Better tolerance was flound at low light level in general, the best was found at sample no. 13145E, both at oxidative and salt stress tests (Fig. 3.).

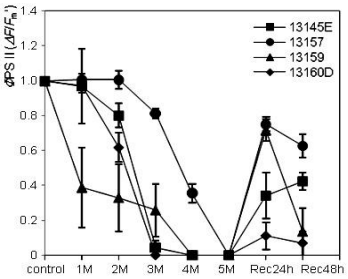

Fig. 3. Example for photosynthetic activity (normalized vert. axis) at 11 µE. Steps on the horizontal axis: control (no salt), increasing concentrations till saturation (5M), recovery in water (Rec 24, 48h).

Conclusion: The Tunisia site probably because of the higher salt occurrence was better and it might contribute in the better tolerance. All samples survived the extereme salt stress and oxidative stresses induced out- and inside of the cell and the aggressive perchloric acid treatments. Their photosynthetic apparatus operated at a promising level both during stress conditions and recovery time. Cyanobacteria and the other organisms in the crusts have delveloped various defences to protect themselves against the reactive oxygen species (ROS). The phototrophic nature of these organisms and their oxygen production under illumination makes it crucial to prevent electron escape to oxygen [7]. Some of these defences are ensimatic (superoxide dismutases, peroxydases, catalases), others are not ensimatic (caroteoids, vitamins, etc.). The effective operation during the stress and survival show that regulating/protecting mechanisms have operated well, the intact crusts (as microecosystems) defensive mechanisms are more efficient than in a single cell.

Strange behavior was observed (similar to our earlier tests in the DLR Mars simulation chamber [1]: samples often produced higher Y (yield, e.g. effective photosynthetic quantum efficiency) after the test than before, except salt stresses. This is probably related to the „hardening", where specific stress psysiological acclimation in the cell happened, suggesting the analysis of hardening related adaptation should be exploited in Mars simulation analysis of extreme organisms.

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References: [1] deVera et al. 2014 IJA 13, 35-44 [2] Kereszturi et al. 2014 LPSC #1357. [3] Radnai 2014. *TDK thesis EKTF*. [4] Marschall et al. 2012 PSS 71, 146-153. [5] Kounaves et al. 2010 *JGR* 115(E7), CiteID E00E10 [6] Hecht et al. 2009 *Science* 325, 64. [7] Latifi et al. 2009 FEMS *Microbiol. Rev*. 33, 258-278.