ADAPTIVE EVOLUTION OF BACTERIA TO HIGH CONCENTRATIONS OF MAGNESIUM SULFATE: IMPLICATIONS FOR EUROPA. A. Yazdani[1], S. Nepal[2], V. F. Chevrier[1], and P. Kumar[3,1,2], [1]Arkansas Center for Space and Planetary Sciences, University of Arkansas, [2]Micro-Electronics and Photonics Department, University of Arkansas, [3]Department of Physics, University of Arkansas, Fayetteville, AR 72701; ayazdani@uark.edu.

Introduction: There are plausible evidences that suggest a global saline ocean exists beneath the icy surface of Jupiter's moon Europa [1, 2]. The ocean might have a redox balance similar to the Earth's oceans [3]. This chemical balance, in the presence of various hydrated compounds, generates a potential habitat at the basal zone of the icy crust where the ice shell acts as a shield against radiation and the temperature is higher (>253 K) [4]. Although high salinity, low temperature and high pressure create extreme environments on Europa, these conditions are not unknown to terrestrial life forms. Bacteria have different ways to adapt to these conditions including regulating the expressions of various genes [5, 6, 7]. The current study provides a quantitative understanding of the effects of long-term exposure to high concentrations of $MgSO_4$, the dominant salt in Europa's ocean [8], and investigates the adaptive strategies used by a model halotolerant bacterium for survival at high concentrations of $MgSO_4$.

Methods: Extreme conditions are non-optimal for mesophilic cells as they usually slow down the cells' growth and a very high magnitude of these conditions may lead to a total collapse of the population. Hence, the region most feasible for the laboratory evolution of mesophilic cells would be when the cells are still able to grow i.e. there is an exponential generation of new cells over rather short amount of time but also feel the selective pressure induced by the new environment, and henceforth called the *tipping region*. The new cells generated under these conditions may undergo genomic and transcriptomic adaptation over the laboratory time-scales.

Our preliminary results from the experiments done on *E. coli* MG1655, a halotolerant gram-negative bacterium, suggest that 15% (w/v) $MgSO_4$ is the tipping salinity where the bacteria can grow and can undergo adaptation to high salt concentration. Once the tipping concentration of salt is characterized, adaptive evolution experiments are performed with this salt concentration using the following strategy. The bacteria were cultured and then inoculated in two tubes of M9 minimal media containing 15% (w/v) $MgSO_4$, under a constant incubation temperature (37°C). The samples are transferred to fresh medium every day (about 6 regeneration time). The morphology of the samples is observed, and the growth rates are measured regularly. The samples are routinely monitored for contamination by performing gram staining. These steps are repeated for 200 passages (~1200 generations).

Results: To examine the adaptation of the cells, we compared the growth rates of the cells from the adaptive evolution experiments and control populations at an elevated level of MgSO4 (20% (w/v)). In Figure 1, we show the growth curves of the control cells and cells from the evolution experiments. These results show that while the control cells are unable to grow in media containing 20% (w/v) $MgSO_4$, the cells that have undergone the adaptive evolution could grow significantly, which implies adaptation to high salt concentration.

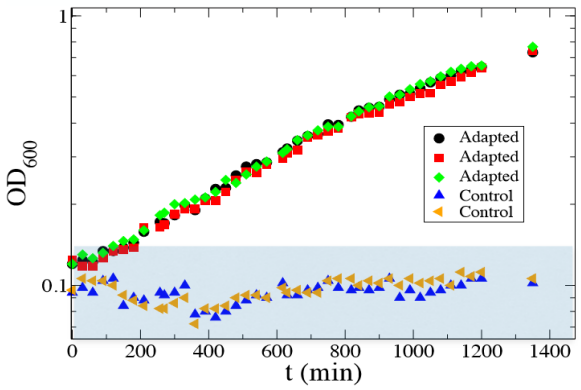

Figure 1. Comparison between the growth curves of the adapted bacteria and the control population in 20% (w/v) $MgSO_4$.

To further understand the nature of adaptation, we study the gene expression of the cultured bacteria by performing Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR). Differential gene expressions of the following genes: fabA, osmC, envZ, corA, cysP, fabR, aqpZ, rpoH, groEL, recA, mreB, ftsZ, were studied using RT-qPCR. GAPDH was chosen as the reference gene and the primers were designed using NCBI Primer-Blast tool. Purity of mRNA and negative control for water contamination were verified. The comparative study was done on three samples—(i) the adapted bacterial cells in M9 Minimal media containing 15% (w/v) $MgSO_4$

(adapted), (ii) control population in the same media (15% control), and (iii) the control population in M9 Minimal media without $MgSO_4$ (0% control). Our results reveal downregulation of corA, cysP and aqpZ genes in the adapted cells (Figure 2).

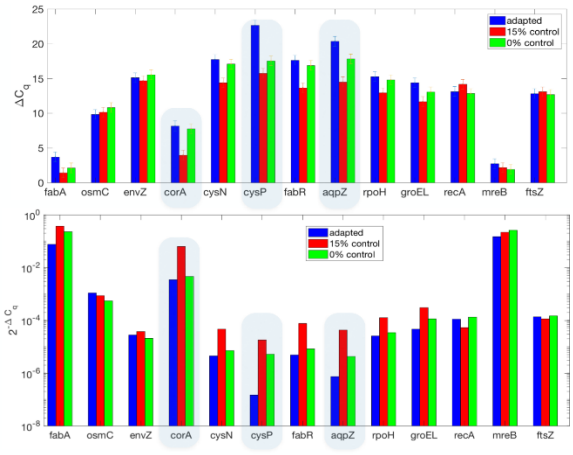

Figure 2. Comparison of gene expressions of adapted and control population of cells at 15% (w/v) $MgSO_4$.

Discussion: Salinity is a limiting factor for bacterial life as the cells thrive to maintain an osmotic balance with the outside environment. Our results suggest that the adaptation to high concentrations of $MgSO_4$ is feasible over laboratory time scales. We further observed that the resultant cells through the adaptive evolution experiments can grow at the salt concentrations where the control cells do not. Furthermore, the comparative gene expression studies using RT-qPCR indicate that the strategy used by the bacterial cells, adapted to high concentration of $MgSO_4$, is to balance the intake of magnesium and sulfate and to prevent the water loss in the cells.

Future work: A better understanding of the adaptation strategies used by the cells at high concentrations will come from a complete transcriptomic profile and investigation of genetic changes. RNA and whole genome sequencing will be performed to investigate the genomic and transcriptomic signatures of adaptation. Furthermore, additional work needs to be done to explore the adaptation to mixture of salts found in Europa's ocean.

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References: [1] Pappalardo R. T. et al. (1999) *J. Geophys. Res., 104 (E10)*, 24015-24055. [2] Kivelson M. G. et al. (2000) *Science, 289*, 1340-1343. [3] Vance S. D., Hand K. P., and Pappalardo R. T. (2016) *Geophys. Res. Letters, 43*, 4871–4879. [4] Marion G. M. et al. (2003) *Astrobiology, 3*, 785-811. [5] Csonka L. N. (1989) *Microbiol. Rev., 53(1)*, 121-147. [6] Berry E. D. and Foegeding P. M. (1997) *J. of Food Protection, 16*, 1583-1594. [7] Marietou A. et al. (2014) *Front. Microbiol., 5*, 749. [8] Kargel J. S. et al. (2000) *Icarus, 148*, 226-265.