

## TOLERANCE OF *BACILLUS PUMILUS* TO PERCHLORATES: IMPLICATIONS FOR MARS’ HABITABILITY

M. Aguirre-Ramírez<sup>1</sup>, P. U. Martínez-Pabello<sup>2</sup>, V. A. López Ruíz<sup>1</sup>, and S. I. Ramírez<sup>3</sup>

On Earth, a study performed on rovers showed that some *Bacillus* species managed to grow in perchlorate solutions. This could mean that certain species of the genus might survive on Mars where perchlorates have been detected. This work explores the tolerance of *Bacillus pumilus* to magnesium perchlorate at a concentration similar to that reported for Mars’ surface.

In 2009 the Phoenix mission identified perchlorate ( $\text{ClO}_4^-$ ) in concentrations close to 0.6% (0.23 M) on the Martian arctic (Hecht et al. 2009). The Curiosity rover and the Mars Reconnaissance Orbiter observed this oxyanion in 2013 and 2015, suggesting a global distribution on the planet. The hygroscopic nature of perchlorate salts might favor the presence of liquid water on the surface of Mars for short-time periods, even at present temperature and pressure conditions, offering a potential habitable niche. More recently, the MARSIS radar of the Mars Express Orbiter detected extensive underground water bodies rich in perchlorates (Orosei et al. 2018). On Earth, a planetary protection study performed on the Mars Science Laboratory showed that some *Bacillus* species managed to grow in perchlorate solutions (Smith et al. 2017). This could mean that certain species of the genus can be targeted as good candidates to evaluate their tolerance to perchlorate concentrations like those reported for Mars. We evaluated the anaerobic growth of *Bacillus pumilus* in nutrient broth (NB) alone, in NB supplemented with 0.25 M magnesium perchlorate ( $\text{Mg}(\text{ClO}_4)_2$ ), and in NB with 0.25 M sodium acetate ( $\text{CH}_3\text{COONa}$ ) and 0.25 M ( $\text{Mg}(\text{ClO}_4)_2$ ).  $\text{CH}_3\text{COONa}$  was provided as an electron donor, while  $\text{Mg}(\text{ClO}_4)_2$  was expected to play the role of the final electron acceptor. Bacterial growth was registered as optical density changes at 630 nm ( $\text{OD}_{630}$ ) for 48 hours. The presence of the chloride anion ( $\text{Cl}^-$ ), an indicator of the anaerobic metabolism of  $\text{ClO}_4^-$ , was argentometrically ( $\text{AgNO}_3$ ) monitored at the beginning and

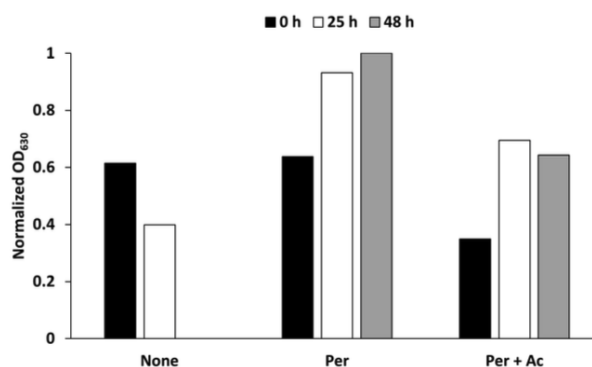


Fig. 1. Growth of *B. pumilus* in (NB)(None), in 0.25 M  $\text{Mg}(\text{ClO}_4)_2$  (Per), and in 0.25 M  $\text{CH}_3\text{COONa}$  with 0.25 M  $\text{Mg}(\text{ClO}_4)_2$  (Per+Ac). The  $\text{OD}_{630}$  changes were normalized to the higher  $\text{OD}_{630}$  value in Per. Incubation conditions: 36 °C, 150 rpm, anoxic environment.

after the 48-hour period. The growth of *B. pumilus* in NB was low, but when  $\text{Mg}(\text{ClO}_4)_2$  was added, significant increments were noticed (Figure 1). This can indicate a certain bacterial tolerance to  $\text{ClO}_4^-$  at a concentration slightly higher to the one reported for the surface of Mars. On the other hand, the addition of  $\text{CH}_3\text{COONa}$  together with  $\text{Mg}(\text{ClO}_4)_2$  does not seem to promote the bacterial tolerance to  $\text{ClO}_4^-$  as the monitored  $\text{OD}_{630}$  changes were smaller than in the essays with only  $\text{Mg}(\text{ClO}_4)_2$ , but slightly better than in NB. No significant changes in the chloride concentration were detected, so we are still not clear about the role of  $\text{ClO}_4^-$  in the bacterial anaerobic respiration processes. We have promising incipient results that deserve a more systematic approach to collect information on the capabilities of *B. pumilus* to metabolize the  $\text{ClO}_4^-$  ion. In this regard, more information about the potential habitability of the present Mars’ surface and subsurface will be gained. We acknowledge funding from CONACyT grant 377887.

### REFERENCES

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<sup>1</sup>Instituto de Ciencias Biomédicas, UACJ Av. B. Franklin 4650, 32310, Juárez, México (marisela.aguirre@uacj.mx).

<sup>2</sup>Instituto de Geología, UNAM.

<sup>3</sup>Centro de Investigaciones Químicas, UAEM (ramirez\_sandra@uaem.mx).