Characterization of the cyanobacterium *Oscillatoria* sp. isolated from extreme sulphureous water from Los Baños de la Hedionda (S Spain)

Elena Martín-Clemente[1], Ignacio José Melero-Jiménez[1], Andreas Reul[2], Miguel Hernández-López[3], Enrique Salvo[1], Elena Bañares-España[1], María Jesús García-Sánchez[1], Antonio Flores-Moya[1]

[1] Departamento de Biologí[3] a Vegetal, Facultad de Ciencias, Universidad de Málaga, Campus de Teatinos s/n, E-29071 Málaga, Spain; [2]Departamento de Ecología y Geología, Facultad de Ciencias, Universidad de Málaga, Campus de Teatinos s/n, E-29071 Málaga, Spain; Departamento de Química Analítica, Facultad de Ciencias, Universidad de Málaga, Campus de Teatinos s/n, E-29071 Málaga, Spain

Introduction

Los Baños de la Hedionda (LH, Málaga, S Spain) is a natural sulphureous (150-200 µM sulphide) spa. Although this high sulphide levels can affect the photosynthetic process (1), there are numerous photosynthetic microorganisms inhabiting the spa (2). Among them, we isolated a strain of the cyanobacterium *Oscillatoria* sp., a genus well known by its tolerance to sulphide (3). The aim of this work was to isolate and to characterize this cyanobacterium, and to analyze the resistance of the isolated strain to sulphide, studied by analyzing the effect of increasing sulphide levels on photosynthetic performance and growth. On the other hand, the limit of genetic adaptation to sulphide was also explored using an evolution experiment named as rachet protocol (4).

Material and methods

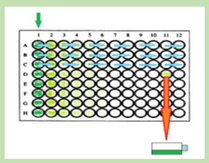

Figure 1. Representation of the SD design.

Isolation and maintenance:

The *Oscillatoria* sp isolation was made by a **successive dilution** (SD) process (Fig. 1). The column A was filled with LH water; the rest of the wells, with 180 µL of BG11. By rows, and successively, a 20 µL aliquot from each well was transferred to the right next one. Following this method, the last well with cells of each row has a population formed by a single cell.

The isolated strain was grown in a 250-mL cell culture flasks, with 100 mL of BG-11 medium buffered with 5 mM HEPES (pH 7). Sulphur (200 µM) was added to the medium every day from a Na_2S- aqueous NaOH master stock solution (pH ~13, 210-240 mM). At pH 7, sulfur is on the form of H2S (50%) and SH- (50%). Flasks were maintained under continuous irradiance of 50 µmol m^{-2} s^{-1}, at 20°C.

Inhibitory Dose (ID) and Lethal Dose (LD)

Inhibitory dose for maximum quantum yield of PS II, on dark adapted cell cultures, (F_v/F_m) was measured using a PAM-2000 fluorimeter (Walz). Measurements of F_v/F_m were carried out after 1 hour incubation in BG 11 plus 20 mM HEPES (pH 7), under different S concentrations (0, 25, 50, 100, 150, 200, 400, 800 µM).

To measure the **LD**, growth rate was measured after five days incubation in the same medium as indicated above at different S concentration (0, 50, 100, 200, 350, 500, 900 µM). *Chla* was used as a biomass estimator. In other experiments, fresh weight was used.

Ratchet protocol: the limit of the genetic adaptation to sulphide

During the initial phase, four replicates of **control** cultures containing growth medium (BG11, 20 mM HEPES, pH 7) plus 200 µM S (mean concentration at LH), and four replicates of **three treatments** with increasing S concentrations were prepared (Fig. 2)

Those cultures were kept seven days and then observed. Cultures showing the same concentration than control ones, were transferred to the next S level (+100 µM). Cultures not reaching a similar biomass to that found in control cultures were not transferred (red asterisks) and maintained in the same S concentration until they reached the control biomass.

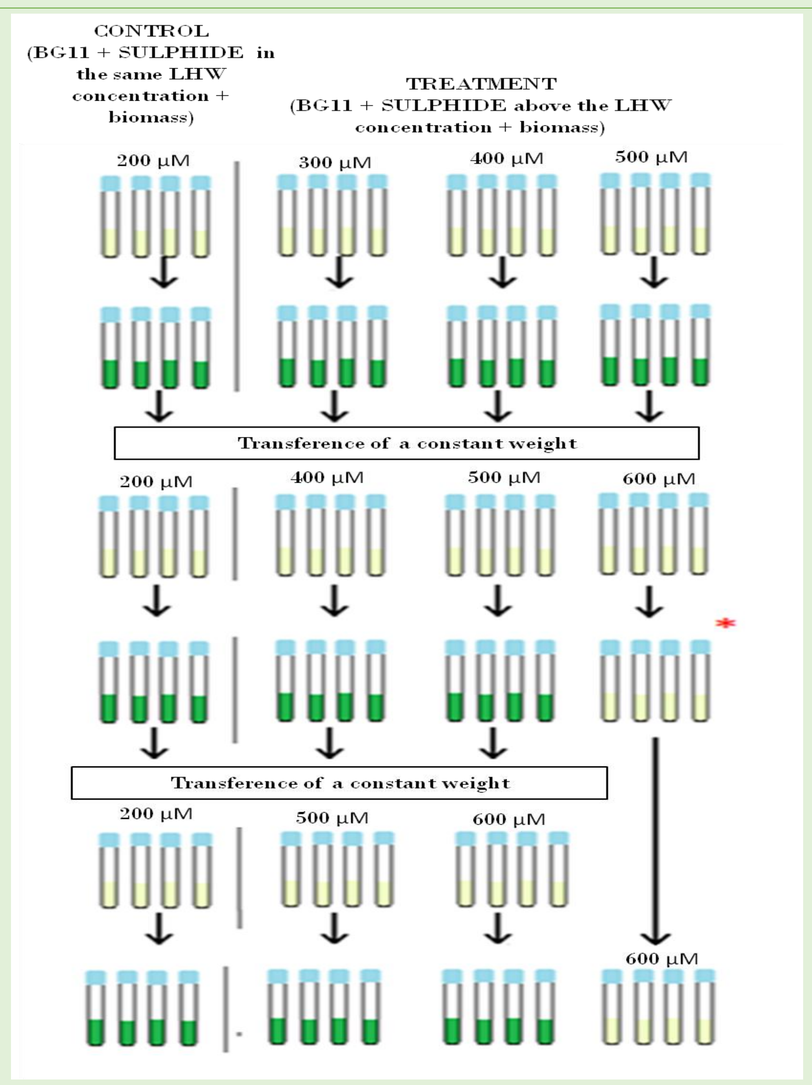

Fig. 2 Ratchet protocol design

Results and Discussion

There were no differences on *Oscillatoria* sp. growth rate (*m*) on the 10-100 µmol · s^{-1} · m^{-2} irradiance range (Fig. 3).

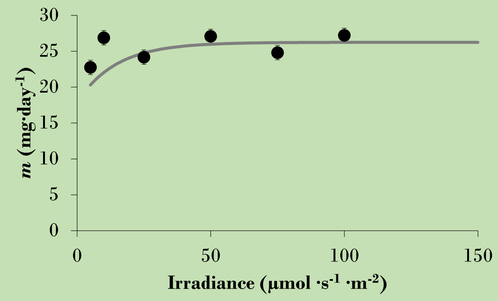

Fig 3 Growth rate (*m*) as a function of irradiance in *Oscillatoria* sp. Data are fitted to a *von Bertalanffy model*. Data are mean ± SD (n= 4)

***Oscillatoria* sp. growth rate (*m*) showed a maximum on the 100-350 µM S range (Fig. 4).**

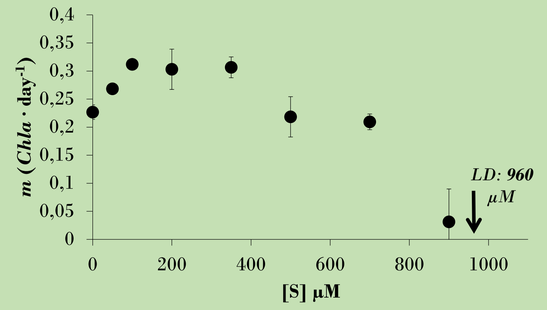

Fig 4 Growth rate (*m*) as a function of S concentration in *Oscillatoria* sp. Data are mean ± SD (n= 4). *LD is indicated on the graph.*

Inhibitory dose for Fv/Fm was much higher for *Oscillatoria* sp. than for a *Microcystis aeruginosa* strain isolated from a non-sulphureous waters.

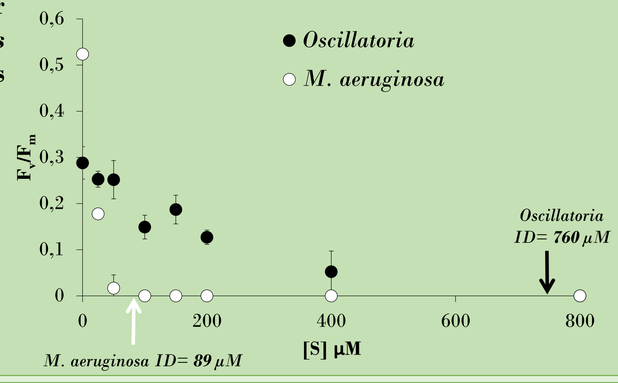

Fig 5 Effect of sulphide on Fv/Fm on dark-adapted cells of *Oscillatoria sp* strain. Data from a cyanobacterium strain isolated from a non-sulphureous waters (*M. aeruginosa*) are shown to compare. Data are mean ± SD (n= 4). *ID are indicated on the graph.*

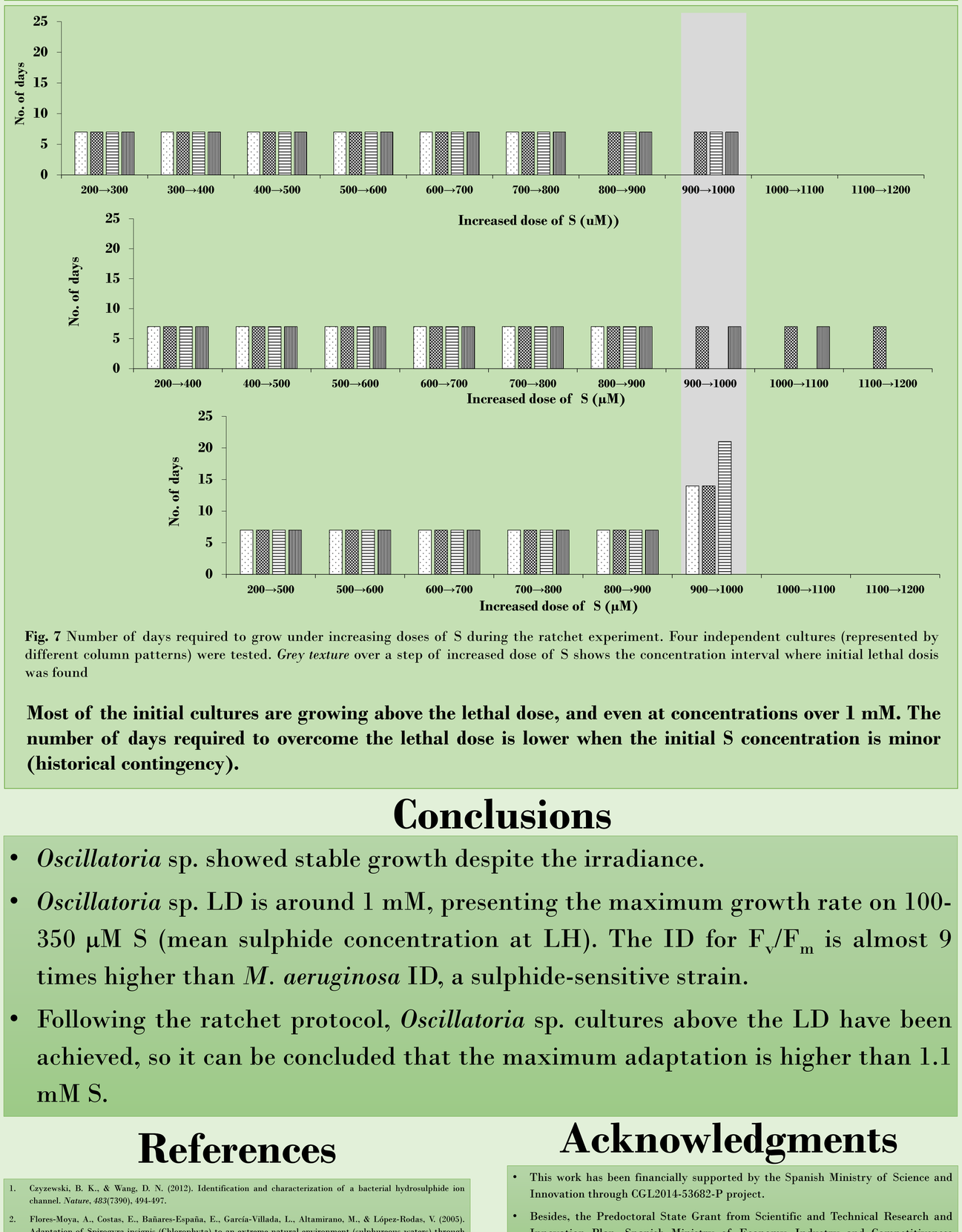

Fig. 7 Number of days required to grow under increasing doses of S during the ratchet experiment. Four independent cultures (represented by different column patterns) were tested. *Grey texture* over a step of increased dose of S shows the concentration interval where initial lethal dosis was found

Most of the initial cultures are growing above the lethal dose, and even at concentrations over 1 mM. The number of days required to overcome the lethal dose is lower when the initial S concentration is minor (historical contingency).

Conclusions

- *Oscillatoria* sp. showed stable growth despite the irradiance.
- *Oscillatoria* sp. LD is around 1 mM, presenting the maximum growth rate on 100-350 µM S (mean sulphide concentration at LH). The ID for F_v/F_m is almost 9 times higher than *M. aeruginosa* ID, a sulphide-sensitive strain.
- Following the ratchet protocol, *Oscillatoria* sp. cultures above the LD have been achieved, so it can be concluded that the maximum adaptation is higher than 1.1 mM S.

References

1. Czyzewski, B. K., & Wang, D. N. (2012). Identification and characterization of a bacterial hydrosulphide ion channel. *Nature*, *483*(7390), 494-497.
2. Flores-Moya, A., Costas, E., Bañares-España, E., García-Villada, L., Altamirano, M., & López-Rodas, V. (2005). Adaptation of Spirogyra insignis (Chlorophyta) to an extreme natural environment (sulphureous waters) through preselective mutations. *New Phytologist*, *166*(2), 655-661.
3. Miller, S. R., & Bebout, B. M. (2004). Variation in sulfide tolerance of photosystem II in phylogenetically diverse cyanobacteria from sulfidic habitats. *Applied and environmental microbiology*, *70*(2), 736-744.
4. Rouco, M., López-Rodas, V., González, R., Huertas, I. E., García-Sánchez, M. J., Flores-Moya, A., & Costas, E. (2014). The limit of the genetic adaptation to copper in freshwater phytoplankton. *Oecologia*, 175(4). doi: 10.1007/s00442-014-2963-1

Acknowledgments

- This work has been financially supported by the Spanish Ministry of Science and Innovation through CGL2014-53682-P project.
- Besides, the Predoctoral State Grant from Scientific and Technical Research and Innovation Plan, Spanish Ministry of Economy, Industry and Competitiveness I+D+i ECC/1402/2013, 2015

Do you want to know more about this experiment?
Get in contact with us.