Disentangling mechanisms involved in the adaptation of the cyanobacterium *Microcystis aeruginosa* to the extreme sulphureous water from Los Baños de la Hedionda (S Spain)

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Introduction

Los Baños de la Hedionda (Málaga, S Spain) is a natural sulphureous spa, where sulphide can reach a concentration of 150-200 µM. Although this ion has biocide properties (1) ,a rich flora can be found in this extreme environment (2). Thus, the adaptation mechanisms allowing resistance of photosynthetic microorganisms to these sulphureous waters were studied using a modified Luria–Delbrück (3) fluctuation analysis. For this purpose, the adaptation of the cyanobacteria *Microcystis aeruginosa* (Kützing) Kützing **Ma1Vc** strain (isolated from a non-sulphureous freshwater reservoir) to La Hedionda waters (LHW) was analyzed, in order to find out if it was achieved by a physiological adaptation process (acclimation) or by the selection of rare spontaneous mutations (genetic adaptation). On this last case, the frequency of the LHW-resistant genetic variant can be calculated.

Material and Methods

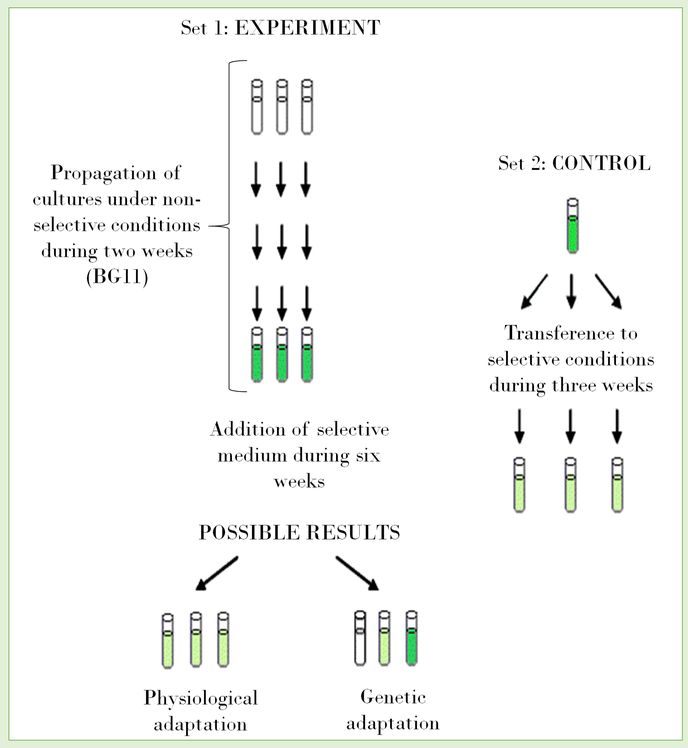
Culture maintenance and sulphide management:

Ma1Vc strain was grown in 100-mL cell culture flasks, with 20 mL of BG-11 medium, under continuous irradiance of 50 µmol m^{-2} s^{-1}, at 20 °C.
To measure the Lethal Dosis (**LD**), sulphur was added every 24 hours from a Na_2S-aqueous NaOH master stock solution(pH ~13, with concentration between 19-23 mM). . HEPES 20 mM was used to maintain the culture at pH 7. At this pH, sulfur (S) are in the forms SH^- (50%) and H_2S (50%) .

Fluctuation analysis (FA):

Two sets were used under the FA (Fig. 1). The fist one (Set 1) started with 90 flasks, inoculated with 10^3 cells. When the cell concentration was above 10^6, the culture medium was changed by LHW (selective conditions) every three days, (150-200 µM). A control (Set 2) was used to measure the experimental error.

At the end of the experiment, cell number in each flask was measured using a hematocytometer.



Mutation rate (μ) and mutation- selection equilibrium (q):
The mutation rate, μ was calculated as:
$\mu = (-\log_e P_o)/(N_t-N_o)$; where N_0 and N_t are the number of cells at the start and at the end of the propagation period (i.e. before the addition LHW), respectively (see Fig. 1). The parameter P_0 was computed as the proportion of Set 1 cultures showing no mutant cells after LHW exposure.
On the other hand, ***q***, the **mutation- selection equilibrium; $q = \mu/(\mu-s)$** is the frequency of the LHW-resistant allele, and ***s*** is the coefficient of selection against this resistant allele, calculated as follows: **$s = 1 - (m^r / m^s)$**, where m^r and m^s are the growth rates on non-selective medium of resistant and sensible strains, respectively. As resistant strain, a 10^6 cells culture from Set 1 was isolated and maintained in the culture collection.

Results and Discussion

The CV of the number of cells between the Set 1 and the Set 2 was significantly different ($p<0.0001$), so the adaptation process that allows resistance was GENETIC.

Table 1:
FLUCTUATION ANALYSIS of LHW-sensitivity to LHW-resistance transformation in the strain Ma1Vc of the cyanobacteria *M. aeruginosa*

	Ma1Vc	
	Set 1	**Set 2**
No. of replicate cultures:	**90**	**40**
No. of cultures with cells in the rank:		
0	73	0
1 - 10^4	1	0
10^4-10^5	1	0
10^5-10^6	6	0
> 10^6	9	40
CV of the no. of LHW-resistant cells per culture	**2.351**	**0.214**
One-tailed Z-test for comparison of CVs	**10.891, $p<0.0001$ (*significant*)**	
Adaptation process	**Genetic**	
µ (mutants $cell^{-1}$ $generation^{-1}$)	**2.1×10^{-7}**	

On absence of selective agent (BG11 medium), the m^s was higher than m^r (Fig. 1). That is because the physiological mutation cost, that decreases the wild-type growing rate. Nevertheless, the m^r was significantly higher on presence of sulfur (100 µM) than the m^s. Indeed, the *resistant LD* was almost three times higher than the *sensible LD* (Fig 2).

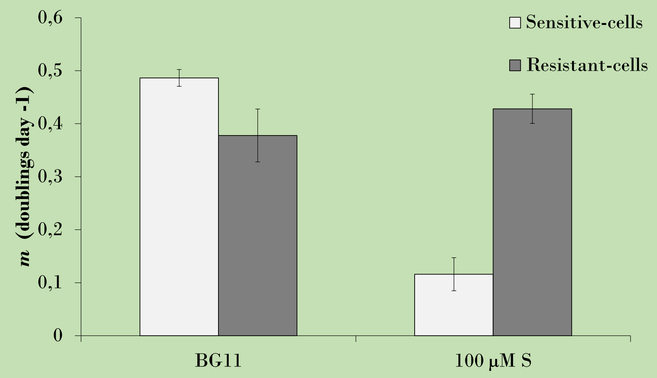


Fig. 1 Growing rate (m) of wild-type LHW-sensitive and LHW-resistant strains under non selective (BG11 medium) and selective (100 µM S) *Data are mean ± SD* (n= 4).

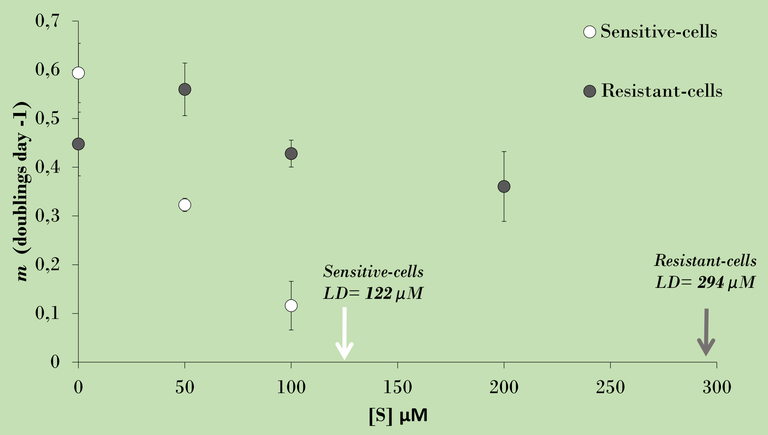


Fig. 2 Growing rate (m) as a function of S concentration on LHW-sensitive and LHW-resistant strain of *M. aeruginosa* Ma1Vc. *Data are mean ± SD* (n= 4). **LD is indicated on the graph.**

Conclusions

- Genetic adaptation was the phenomenon that allowed Ma1Vc strain to survive on sulphide mediums, with a mutation rate **2.1×10^{-7} cell division**. The mutation-selection equilibrium was **9.4×10^{-7} cells**.

- It could be hypothesized that this cyanobacterium could adapt to sulphureous environment by the selection of favored mutants.

Using both m^s and m^r growing rates on BG11, a coefficient of selection (s) of 0.222 was computed. The estimation of the frequency (q) of LHW-resistant allelles in wild-type populations of Ma1Vc, was calculated to be 9.4×10^{-7} cells.

References

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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

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