THE LIMIT OF SULPHIDE ADAPTATION IN THE SULPHIDE-SENSITIVE CYANOBACTERIUM *Microcystis aeruginosa*

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Although sulphide has played an important role in the evolution of photosynthesis, it produces a lethal effect on most photosynthetic organisms due to its redox activity on certain enzymes, inhibiting oxygenic photosynthetic and respiratory electron transport. However, cyanobacteria vary in sulphide tolerance, showing different degrees of sulphide resistant oxygenic photosynthesis and even some taxa have the capacity to perform anoxygenic sulphide-dependent photosynthesis. *Microcystis aeruginosa* is a sulphide-sensitive species. However, it is known that genetic adaptation of aquatic photosynthetic microorganisms to selective agents can be rapidly achieved, even at lethal levels, as the consequence of single mutations. The aim of this work was to determine the maximum sulphide concentration to which this sulphide-sensitive species is able to adapt in order to shed light on the process of sulphide adaptation in cyanobacteria.

We used three *M. aeruginosa* strains, Ma1Vc, Ma5Vc and MaAVc, isolated from a non-sulphureous environment, and whose lethal doses were 0.10, 0.16 and 0.20 mM sulphide, respectively. To study the adaptation of these strains to sulphide a modified ratchet experiment was carried out. We maintained large populations in order to ensure the occurrence of rare spontaneous mutations (that can possibly confer adaptation) and a strong selection pressure (in order to fix such mutations within the population). Cultures were exposed to increasing concentrations of sulphide at three different selection levels (each level started with a different sulphide dose). After 7 days of culture, cell concentration in sulphide-enriched cultures was compared to that in control cultures (without sulphide). If treated cultures showed a similar concentration to that observed in controls, they were transferred to the next ratchet cycle, i.e. to a higher sulphide concentration. A population was considered to have reached the maximum capacity of adaptation to sulphide when no further cell growth was observed after 90 days of exposure to this sulphide concentration.

The *M. aeruginosa* strains reached different limits of sulphide adaptation. Ma5Vc and Ma1Vc strains adapted up to 0.40 mM sulphide, i.e. 2,5-fold and 4-fold their initial lethal doses, respectively. However, the MaAVc strain, which showed an initial higher sulphide tolerance, adapted up to only 0.27 mM. Sulphide-lethal dose and photosynthetic performance of the resistant strains obtained in the ratchet experiment were characterized. The lethal dose of the *M. aeruginosa* Ma1Vc and Ma5Vc mutant strains was 0.40 and 0.72 mM sulphide, respectively. These resistant strains showed lower growth and photosynthetic rates than wild-type ones in the absence of sulphide, which indicates the physiological cost of the mutation conferring sulphide resistance.

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