Phosphorylation of MAP Kinases crucially controls the response to environmental stress in *Dunaliella viridis*

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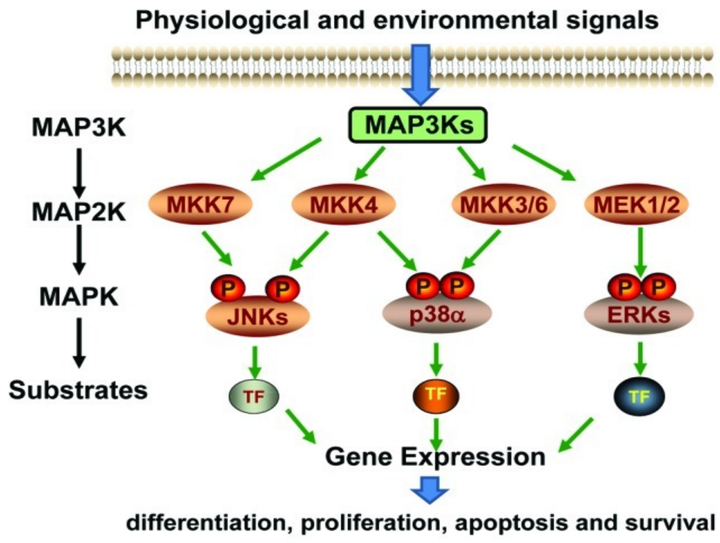
INTRODUCTION

Dunaliella viridis (*D. viridis*) is an unicellular green microalgae that can adapt to a wide variety of environmental stress conditions. Closely related to the build-up of stress responses, MAPKs (*Mitogen Activated Protein Kinases*) are highly conserved serine/threonine kinases that convert extracellular stimuli into a wide range of responses.



In eukaryotic cells, MAPKs are involved in both cell proliferation and differentiation (ERK pathway) and stress responses (JNK and p38 pathways), through protein kinase cascades

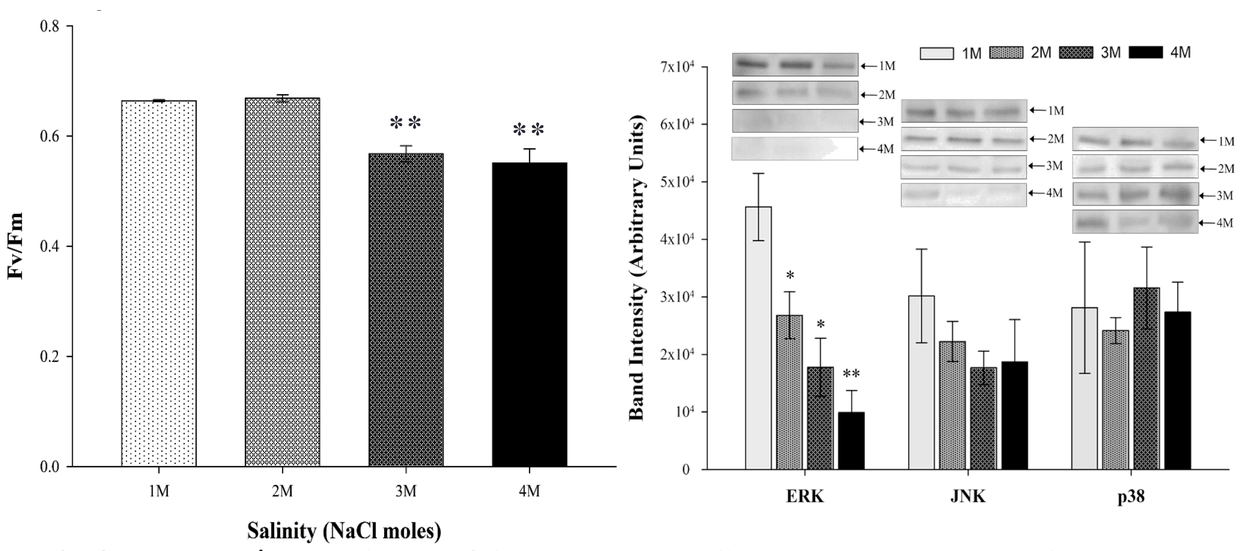
The experiments were designed to explore the possibility that these lower organisms respond to sudden changes on the environmental conditions by activating MAPK signaling pathways such as p38 and JNK, while deactivation of ERK would reduce cell proliferation. Inhibiting these pathways would compromise the ability of this microalga to acclimate to the new prevailing conditions, thus leading to cell death.

MATERIAL AND METHODS

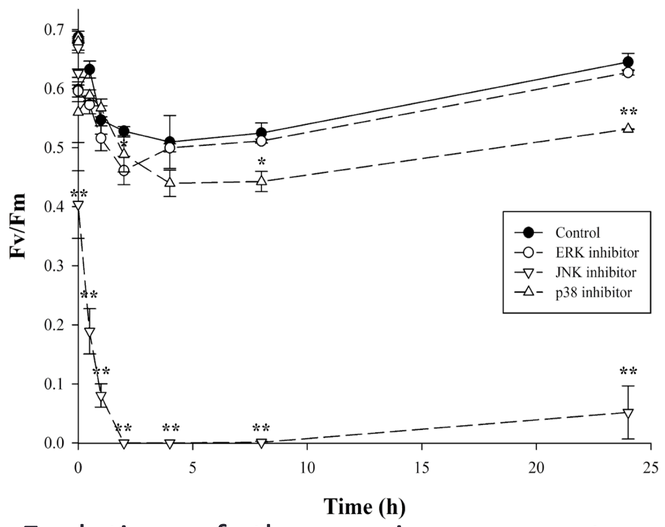
The ability of *D. viridis* to survive at increasing salinities was estimated by growing the cells at 1, 2, 3 and 4 M NaCl for a period of several weeks. To study the acclimatization process under different stress situations, cells were exposed to three types of non-lethal stress: (**i**) **hyperosmotic stress**, (**ii**) **high irradiance**, and (**iii**) **UV radiation.**

To test the role of each MAPKs, specific inhibitors of each of these three MAPKs were used. At fixed times, aliquots were collected to see the evolution of the phosphorylation of these MAPKs over time (24 hours), and its effect on cell viability, measured with the maximum quantum yield of Photosystem II fluorescence (*Fv/Fm*). MAPK immunodetection was carried out using antibodies against the phosphorylated forms of mammalian p38, ERK and JNK proteins (Ph-p38 #9211, Ph-JNK #9251, Ph-p44/p42 #9106; Cell Signaling Technology, Beverly, MA, USA).

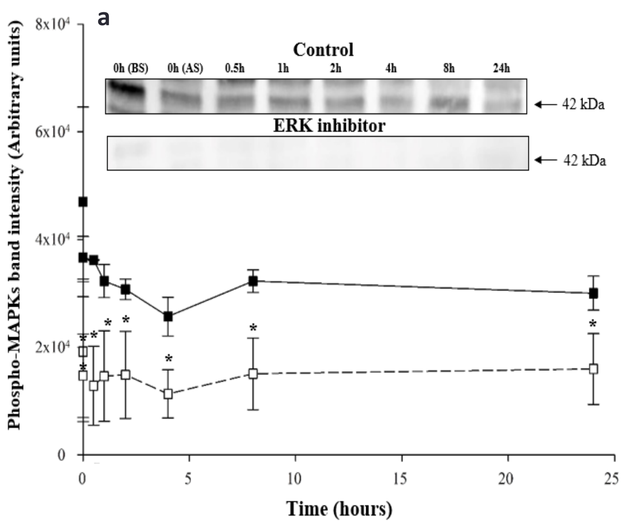
RESULTS



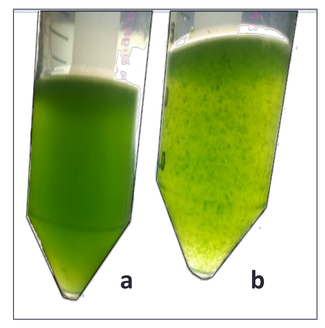
Left figure: Fv/Fm values of long-term acclimated *D. viridis* cultures at increasing salinities. Cultures at 2M, 3M and 4M NaCl were compared against 1M NaCl treatment (control). Right figure: Band intensity (in relative units) of the three MAPK-like proteins (ERK, JNK and p38) of the same cultures.



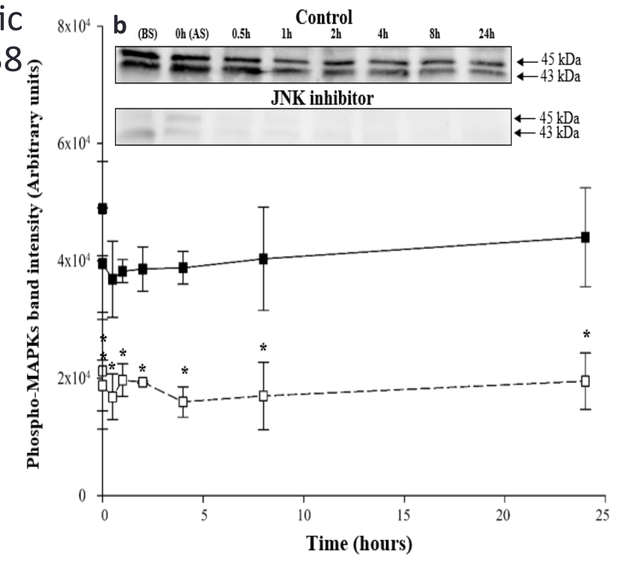
Evolution of the maximum quantum yield of *D. viridis* after high irradiance shock (as a representative example) in the presence or absence of the specific inhibitors of the ERK, JNK and p38 cascade.

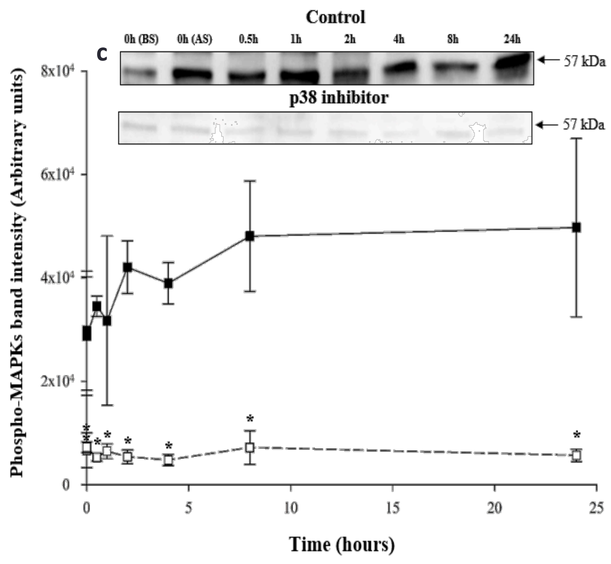


Time course (24h) of the phosphorylation level of ERK-like (a), JNK-like (b) and p38-like (c)the different MAPK-like proteins after hypersaline stress (as a representative example), both in control cells (stress without inhibitor) and in the cells stressed after 2 h incubation with the corresponding inhibitors.



50 mL Falcon conical tubes showing the effects of the JNK cascade inhibitor 4 hours after hypersaline stress (b) in comparison to the control, subjected to the same stress, but without JNK inhibitor (a)





CONCLUSIONS

- ✓ There were significantly **lower phosphorylation** levels of **ERK-like proteins** in the cultures subjected to the maximum NaCl concentration (**4M**), and no significant differences in the phosphorylation levels of JNK-like and p38-like proteins between cultures.
- ✓ After stress exposure, there were **higher phosphorylation** levels in **JNK-like** and **p38-like proteins** after stress exposure, showing their activation, and **lower phosphorylation** levels of **ERK-like proteins** after stress exposure, not being significantly different when compared to the control.
- ✓ It could be estimated the essential role played by **JNK-like proteins** in the **maintenance of cell viability** after an environmental stress situation, and the involvement of **p38-like proteins** in the **acclimatization process**, while **ERK-like proteins** appear to control the **cell division process**, not being directly involved in the acclimatization process.

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