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Targeted and non-targeted metabolomic analyses of *Heterocapsa cf. bohaiensis* under nickel and iron high concentration and its photophysiology

Meriot V.^{1,2}*, Herbette G.³, Garayev E.⁴, Coulombier N.⁵, Le Déan L.², Jauffrais T.², Lebouvier N.¹

1. ISEA EA7484, Univ. Nouvelle-Calédonie, 98851 Nouméa, Nouvelle-Calédonie, France ; 3. CNRS, Centrale Marseille, FSCM, Spectropole, Aix Marseille Université, Campus de St Jérôme-Service 511, 13397 Marseille, France ; 4. Aix Marseille Univ, CNRS 7263, IRD 237, Avignon Universite, IMBE, 27 Blvd Jean Moulin, Service of Pharmacognosy, Faculty of Pharmacy, 13385 Marseille, France ; 5. ADECAL Technopole, 1 Bis Rue Berthelot, 98846 Nouméa, Nouvelle-Calédonie, France

Introduction

- □ New Caledonian coastal waters are naturally rich in metallic trace elements. (Biscéré et al. 2017; Merrot et al. 2022)
- An excess of metallic inputs induce an **oxidative stress.** (Pinto et al. 2016; Danouche et al 2022)



U Heterocapsa cf. bohaiensis, a dinoflagellate isolated in New Caledonia, is associated with shrimp and crab mortalities. (Xiao et al. 2018)

□ Might be a good candidate to study the production of **metabolites** of **interests**

Materials & Methods

- **Culture in photobioreactors:**
- Continuous culture of *H*. cf bohaiensis
- Addition of **nickel** and/or **iron** after steady state
- **Experimental conditions (in triplicate):**
- **Metals:** Ni 10⁻³ M ; Fe 10⁻³ M ; Ni+Fe 0.5 10⁻³ M (of each)
- **Times:** Control t_0 ; t_{24h} ; t_{72h} ; t_f (13 days)
- **Analyses:**



(e.g. carotenoids, biotoxins, fatty acids...)

Does the species adapted to this particularly metal-rich environment and does it impacted the **photosystem** and **metabolome** by developing **adaptive strategies**?

> Aim: Study the impact of nickel & iron on the photosystem and metabolome

Chlorophyll a fluorescence using PAM

(Pulse Amplitude Modulated) fluorometry

- **Bioaccumulation** capacity by ICP–MS/OES
- **Pigment** analyses through HPLC-UV (450 nm)
- **Fatty acids -** GC-MS (Transesterification)
- Non targeted metabolites NMR

Results



Photosynthetic efficiency

Maximum Quantum yield

efficiency > Maximum photosynthetic by **25%** and **15 %** in Fe decreased and Ni+Fe condition, respectively







H. cf. bohaiensis in 10L photobioreactor

Identification of typical dinoflagellate pigments

Pigment analyses

> Increase of diatoxanthin concentration by 3 after 13 days of exposure to iron at

> Negative impact on photosynthetic activity due to high iron concentration

OJIP tests

efficiency > Decrease of quantum parameters after 13 days with iron

Absorption, trapping and dissipation were higher with addition of iron (concentration dependant) after 13 days

Bioaccumulation capacity



10⁻³ M

Fatty acids

- Increase over time in **nickel** condition
- \succ **Increase** from t₀ to t₇₂ and **decrease** at t_f, with iron
- Decrease over with both time metals

NMR¹H

- > Detection of major metabolites
- > Higher fatty acids content than pigments
- Detection of carbohydrates
- Some metabolites have to be identified

Discussion

Photosynthetic efficiency

> H. cf. bohaiensis is tolerant to nickel at high Increase of concentrations diatoxanthin in iron condition

In Iron conditions:

> Fv/Fm - H. cf. bohaiensis is impacted after a long term exposure

> **RLC** - Iron reduced the ability to use light > OJIP - Iron stimulates capture and absorption of light energy & slows down the electron transport rate between photosystem II & I > Increase of specific fluxes (absorption, trapping and dissipation)

Pigment analyses

Responsible of

dissipation

> Photosystem

protection against

increase

Fatty acids

- \succ Majority of 3 fatty acids more or less affected by the presence of metals NMR¹H
- > Majority of **fatty acids** > Detection of other metabolites than in targeted methods

Bioaccumulation

iron induced **ROS** Greater bioaccumulation of iron than nickel Transfer in the food chain (Hédouin et al. 2010) (Huang et al. 2017)

Conclusion

Aim: Study the impact of nickel & iron on the photosystem and metabolome

- ✓ **Toxic** effect of iron dependant of the concentration **on the photosystem**
 - ✓ Iron stress adaptative strategies to protect against ROS

✓ Risk of **metal bioaccumulation** in the **food chain**

- ✓ Identification of specific metabolites by targeted metabolomics
- NMR results show metabolites previously metabolites and new ones
- □ Untargeted metabolomic data have to be statically analysed to evaluate the metabolome response to metals (NMR & UPLC-HRMS)

References

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