Characterisation of a secondary carotenoid producer microalga of the genus *Coelastrella*

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INTRODUCTION

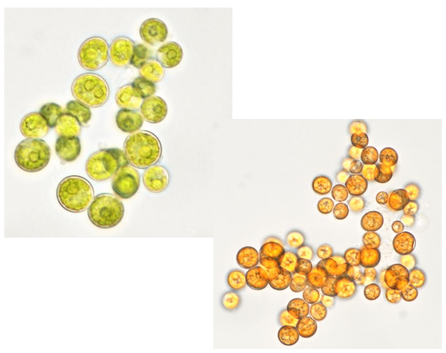
Some green microalgae synthesize secondary carotenoids as protecting agents under stress. These pigments have high value as feed supplement for aquaculture and as health products. The most promising pigment is astaxanthin, because of its antioxidant, antitumoral and anti-inflamatory properties. The most used natural source of this pigment is the microalga *Haematococcus pluvialis*. However this species grows slowly and lacks robustness for easy cultivation. Therefore, other species are investigated for astaxanthin production. Here, we isolated a local microalgal strain that is a natural producer of secondary carotenoids. We identified it and analysed culture conditions leading to secondary carotenoid accumulation.

IDENTIFICATION

Molecular biology

We sequenced the Internal Transcribed Spacer 2 (ITS2) and the 18S *[1]*. We used a nucleotide BLAST (on NCBI) to identify the strain and the SINA program (Silva) to confirm it. These methods lead us to classified the strain as ***Coelastrella* sp.**

***Figure 1**: Optical micrographs of green and orange-red cells*



Morphological aspect (by Microscopy and Scanning Electron Microscopy (SEM))

The isolated strain is a green microalga, easily cultivable in the laboratory. It is constitued of spherical-shaped (ϕ ≈ 10µm), non-mobile cells with a single starred plastid with numerous lobes and a large pyrenoid (*Fig. 1*). After ≈20 days of culture, the strain turns from green to orange-red color (*Fig. 1*). The SEM micrographs allowed us to observe that the cells have **meridional ribs** that converge at two poles of the cells (*Fig. 2*). These ribs are a tyical characteristic of *Coelastrella* genus *[1,2]*.

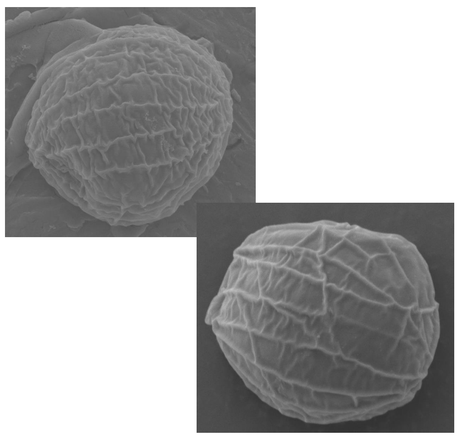


***Figure 2:** SEM micrographs of green and orange-red cells*

GROWTH

Generation times in different conditions were obtained from optical density measurements during growth in 250 mL flasks using Bold-3N medium.

Autotrophic growth

Table 1 shows the effects of light intensity and CO_2 supplementation on generation times for autotrophic cultivation. Growth appeared strongly limited by CO_2 on air. CO_2 supplementation (5 %) led to fast growth with maximum rate around 400 µmol photons.m^{-2}.s^{-1}.

Heterotrophic growth

Among different carbon sources, the microalga was only capable of assimilating glucose in darkness. The generation time on glucose (10 g.L^{-1}) was lower than in any autotrophic condition tested but the growth continues for longer times (data not shown).

Conditions		Generation time (h)
Carbon source	Light intensity[1]	
Autotrophy (in flasks)		
Without	100	27,64 ± 3,58
	200	22,03 ± 3,15
	400	23,8 ± 1,47
With CO2	100	18 ± 2
	200	14,6 ± 0,3
	400	12,9 ± 0,8
Heterotrophy (in flasks)		
Glucose	0	56 ± 3

[1] *in µmol of photons.$m^{-2}s^{-1}$*

***Table 1:** Generation times in different conditions.*

PIGMENT CONTENT

Stress conditions

To accelerate carotenogenesis, we applied a stress which was a combination of **nitrogen starvation and exposure to high light intensity** (>500 µmol photons.m^{-2}.s^{-1}). This led to complete cell reddening after 5 days, compared to ≈20 days without any stress applied.

Pigments analyses

The pigment content was analysed by reverse HPLC (CORTECS C18 Column, 90Å, 2.7 µm, 4.6 mm X 150 mm, 1/pkg).

- **Pigments from green cells**

Usual pigments were found in the green (unstressed) cells (*Fig. 3).*

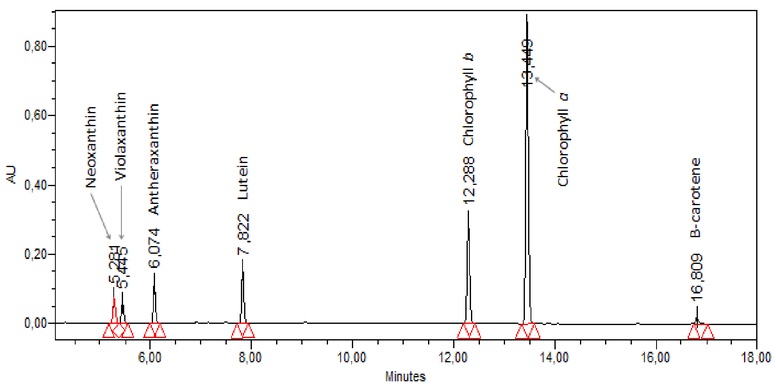


***Figure 3:** HPLC chromatogram (430 nm) of a pigment extract from green cells*

- **Pigments from orange-red cells**

Pigments from stressed cells were first subjected to saponification in order to de-esterify secondary carotenoids *[3]*.

We applied stressful conditions during 4 days to autotrophic and heterotrophic pre-cultures. A typical chromatogram (obtained for heterotrophically-grown cells) is presented in *Fig. 4*. This chromatogram shows several secondary carotenoids, among which astaxanthin and cantaxanthin, as well as non-identified cars.

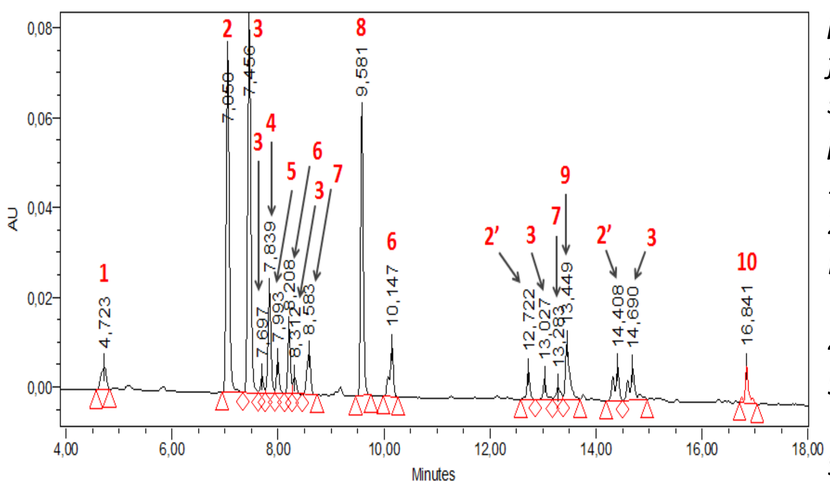


***Figure 4:** Chromatogram of a pigment extract (430 nm) from orange-red cells synthetized after a heterophic pre-culture.*
1 – Chlorophyllide a
2 – Free astaxanthin, 2'-Esterified astaxanthin
3 – Unknown carotenoid
4 – Lutein
5 – 6 - 7 Unknown carotenoids
8 – Cantaxanthin
9 – Echinenone
10 – ß-carotene

- **Effect of pre-culture conditions on pigment content**

After 4 days of carotenogenesis, astaxanthin biomass content was found higher for autotrophic pre-cultures, whereas cantaxanthin content was not significantly dependent on pre-culture. Moreover, higher amounts of chlorophylls were found for autotrophic pre-cultures, which must be due to inhibition of their synthesis in darkness.

Pigments	Autotrophic pre-culture	Heterotrophic pre-culture
Chlorophyll a	2,2 ± 0,2	0,89 ± 0,03
Chlorophyll b	0,77 ± 0,06	0,335 ± 0,003
Astaxanthin	1,41 ± 0,05	1,07 ± 0,04
Cantaxanthin	0,87 ± 0,09	0,97 ± 0,05

***Table 2:** Pigments content in different conditions. The contents are expressed in mg.g^{-1} of dry weight (average ± standard deviation in 3 experiments).*

CONCLUSION

In this study, we first identified a locally isolated strain as *Coelastrella* sp. that is a secondary carotenoid producer. A known typical feature of this genus, that we could observed in the strain by scanning electron microcopy, is the presence of meridional ribs. This strain grows both autotrophically and heterotrophically and is able of fast change in pigment composition under controlled stress conditions. A variety of secondary carotenoids accumulate, among which astaxanthin, cantaxanthin and echinenone. Unidentified compounds will be further analyzed by mass spectrometry.

REFERENCES

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