

## Culturing a novel strain of *Haematococcus pluvialis*, LSBB612 (ALG\_App006)

### Background

The genus *Haematococcus* is found globally, with reports of isolates from all continents with the exception of Antarctica, with hostile areas of isolation including the arctic circle (Klochkova *et al.*, 2013). *H. pluvialis* is of commercial interest due to its ability to produce copious amounts of astaxanthin, reaching up to 5 % dry weight in the encysted aplanospore state (Wayame *et al.*, 2013). Astaxanthin is sold as a pigment for aquaculture and in animal feed, and is marketed as an antioxidant for the nutraceutical market. The *H. pluvialis* derived astaxanthin industry is commercially successful; however, several constraints are ever-present including issues of contamination and grazing, high extraction costs, high light requirements for encystment, and conversely, photo-bleaching (Shah *et al.*, 2016).

Astaxanthin is produced under high light and nutrient deplete conditions (García-Malea *et al.*, 2008). High temperature is rarely implemented to induce astaxanthin production, as it was reported to severely reduce biomass yield, and thus decrease astaxanthin productivity (Tjahjono *et al.* 1994). Currently the red stage of astaxanthin production is constrained by biomass production in the green stage, which requires strictly controlled culture conditions. Optimal reported temperatures for the vegetative growth of *H. pluvialis* are between 20 and 28°C (Wan *et al.*, 2014), with temperatures in excess of 30°C shown to induce transition from the green vegetative stage to the red stage with the formation of aplanospores. Domínguez-Bocanegra *et al.*, (2004) demonstrated optimal growth at an irradiance of 177  $\mu\text{mol photons/m}^2/\text{s}$  with higher density cultures achieved under continuous light.

### Aim

To demonstrate the growth characteristics of the novel *H. pluvialis* strain LSBB612 with an unusually high temperature optimum using the Algem lab-scale photobioreactor.

### Experimental Design

*H. pluvialis* LSBB612 starter cultures were grown under 40  $\mu\text{mol photons/m}^2/\text{s}$  continuous red/blue (3:1) light in TAP (*Chlamydomonas* Resource Centre) + B12 medium (0.001 mg/L cyanocobalamin), with aeration at 15  $\text{cm}^3/\text{min}$  5%  $\text{CO}_2$  in air, shaking at 120 rpm. Algem flasks (1L) containing 400 ml TAP + B12 medium were inoculated at  $4 \times 10^4$  cells/ml. Flasks were cultured in the Algem in duplicate at 20, 25 or 30°C, 100  $\mu\text{mol photons/m}^2/\text{s}$  continuous light (85:15 white/red mix), intermittent aeration, with 5%  $\text{CO}_2$  to maintain pH 7, and agitation at 120 rpm.

## Results

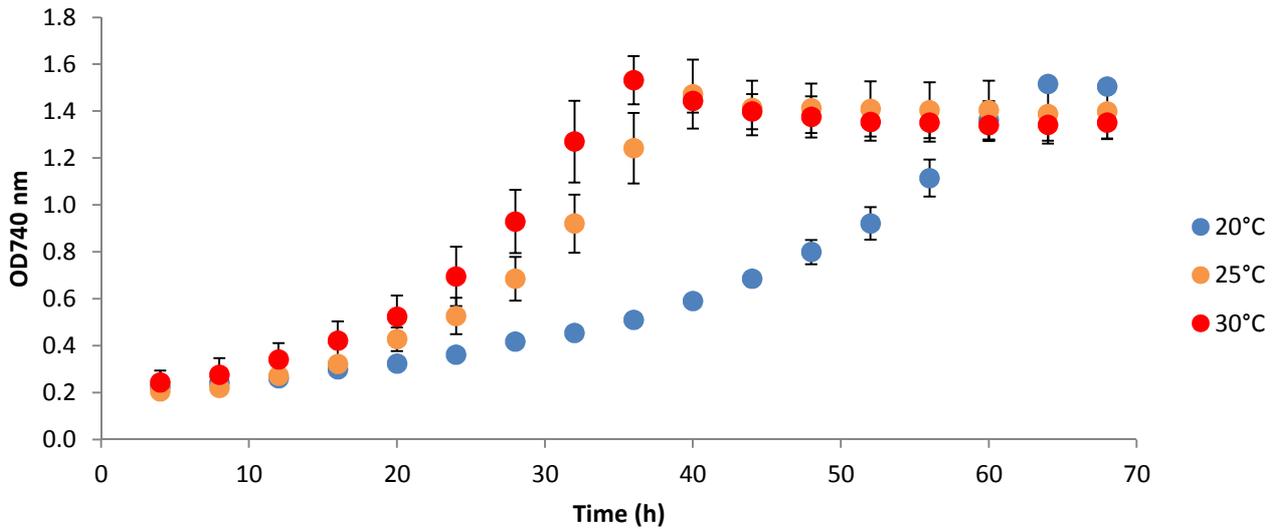


Figure 1 - *H. pluvialis* LSBB612 growth under different temperatures (20°C, 25°C and 30°C)

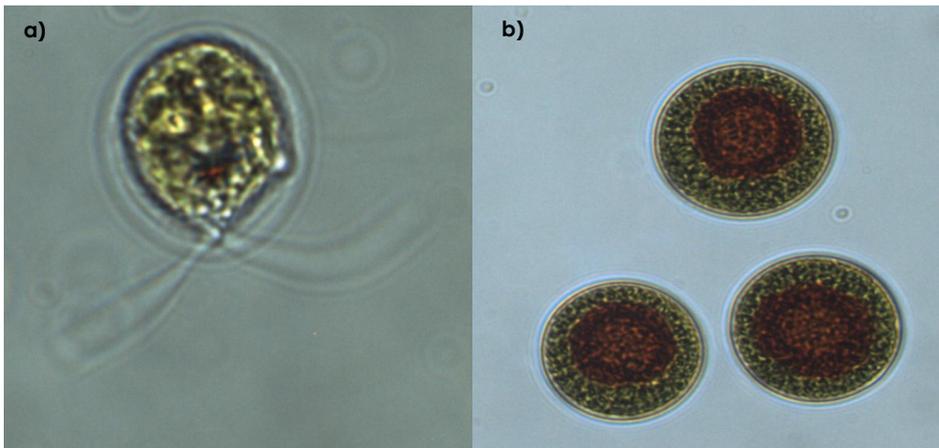


Figure 2 - a) motile macrozooids and b) palmelloids observed during growth

## Discussion

*H. pluvialis* LSBB612 is classified as non-motile according to the categorisation of Han et al. (2012) as the culture predominates in the palmelloid form rather than the green motile macrozooid. In this experiment it was shown that under the conditions tested, 30°C resulted in the highest growth rate, attaining a maximum doubling time of ~12 h. Growth was shown to plateau for all temperatures at a similar OD due to acetate depletion. This strain of *H. pluvialis* offers commercial interest as it is able to tolerate temperatures greater than conventional culture conditions (Wan et al., 2014) and did not encyst during the whole culturing period. A mixture of green motile macrozooids and green palmelloids were maintained. Further experiments will be conducted to ascertain the upper-temperature limit for vegetative growth of this strain.

## References

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