Our research

Arsonolipids in brown algae *Ectocarpus*

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**Introduction**

- Seaweed is growing in popularity, e.g. for cooking, snacking, as a food supplement and as well in various skincare products.
- Seaweed is high in nutrients, such as minerals and vitamins, however, can have high total arsenic (totAs) conc.
- The majority is on the form of arsenosugars, with low percentage of toxic inorganic arsenic (iAs), Figure 2.
- Arsenolipids (AsLps) have recently been found in seaweeds, Figure 1.
- Arsenic accumulates in seaweeds since phosphate transporters take up arsenate (As) in addition to the phosphate.
- Biosynthetic pathway for the formation of arsenolipids has been suggested but not proven.

**Aim and hypotheses**

- Grow *Ectocarpus* (EC) from single cell and compare to naturally growing *Ectocarpus* and related algae.
- Study the dependence of AsLps and AsSugars on environmental conditions such as nutritional status and oxidative stress.
  - Will nutrient deficient seaweed conserve N or P, e.g. by replacing choline in phospholipids (PL) with arsenosugar?

**Materials and methods**

- Seaweed samples extracted in methanol/dichloromethan (1:2) and AsLps separated on reverse phase (RP) C18 column and introduced simultaneously to ICP-MS and ESI-MS (orbitrap).
- Extracted in water and AsSugars measured on HPLC-ICP-MS with anion exchange column.
- totAs in residue determined.

**Sample design**

- Three strains (S1-S3) of *Ectocarpus* grown at:
  - Ctrl: Control (1/2 Provasoli)
  - N: Low nitrate (1/3 N of ctrl)
  - P: Low phosphate (1/3 P of ctrl)
  - OS: Oxidative stress (+ H2O2)
  - S3 also grown +2 pb arsenic.
  - As conc. in provasoli media 0.8 pb.

**Conclusions**

- The concentration of AsLps is similar in EC cultures and EC nature, Figure 5.
- Majority of arsenic in cultures is non-extractable, Figure 5.
  - Bound in membranes?
  - AsHC360 is the main AsLp in EC cultures and EC nature.
  - Good repeatability between 3 replicate cultures, Figure 6.
  - Increased production of AsLps under stress for S1-S2 (not shown).
  - Additional arsenic in media leads to higher production of AsHCs, Figure 6 a) and b).
- For low phosphate conditions there is a significant increase in production of AsPL (S3) but no AsSugars containing phosphate were present (S1-S3), Figure 6.

**Results**

- The totAs was determined in all fractions: lipid soluble (LS), water soluble (WS) and residue (RS), Figure 5.
- AsLp and AsSugar profiles quantified with ICPMS and identified with ESIMS, Figures 7.

**Figure 1. Examples of arsenolipids (AsLp)***

**Figure 2. Toxicity profile of seaweed.***

**Figure 3. Instrumental setup**

**Figure 4. Schematic of the sample design**

**Figure 5. Distribution between water soluble arsenic species (WS), lipid soluble arsenicals (LS) and the arsenic still bound in the residue (RS) after sequential extraction for: a) Ectocarpus species found in natural habitat, includes the total arsenic (totAs) c) Ectocarpus cultures. Also a close up of the LS fraction: b) Ectocarpales d) cultures. Error bars represent standard deviation.***

**Figure 7.** a) Arsenolipid profiles and quantification for S3. Condition statistically significant from ctrl using ANOVA: *P<0.05, **P<0.001* b) Arsenolipid profiles and quantification for S3 at enriched arsenic conditions. c) AsSugar profile and quantification for S3 + As.

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 Fraction: 
- WS: Water soluble
- LS: Lipid soluble
- RS: Residue

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**References**


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