Our research – your profit

Arsenolipids in brown algae *Ectocarpus*

Ásta H. Pétursdóttir[1,2,3], Kyle Fletcher[3], Helga Gunnlaugsdóttir[1], Frithjof Kuepper[3] and Jörg Feldmann[2]

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 seaweed since phosphate transporters take up arsenate (iAs) in addition to the phosphate.
- Biosynthetic pathway for the formation of arsenolipids has been suggested but not proven.

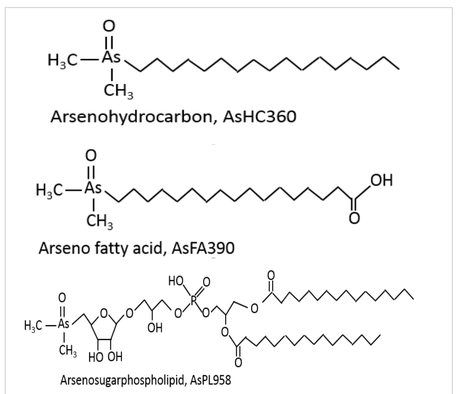

Figure 1. Examples of arsenolipids (AsLps)

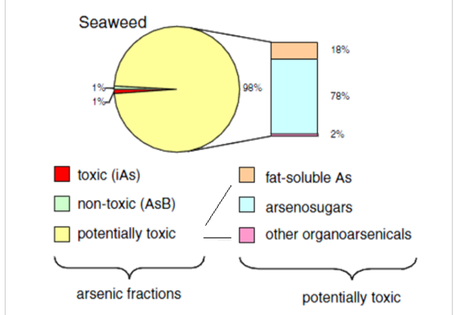

Figure 2. Toxicity profile of seaweed.
Ref: Feldmann and Krupp. 2011. Anal Bioanal Chem.

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 seperated on reverse phase (RP) C18 column and introduced simultanously to ICP-MS and ES-MS (orbitrap).
- Extracted in water and AsSugars measured on HPLC-ICP-MS with anion exchange column
- totAs in residue determined.

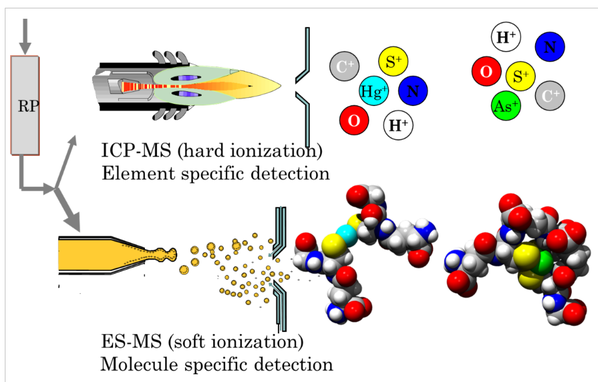

Figure 3. Instrumental setup

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 (+ H_2O_2)
- S3 also grown +2 ppb arsenic.
- As conc. in provasoli media 0.8 ppb.

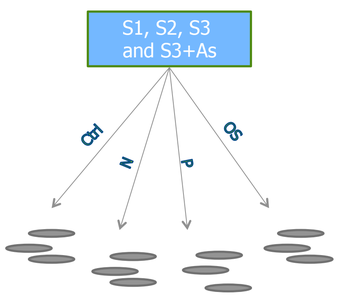

Figure 4. Schematic of the sample design

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 AsPLs (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.

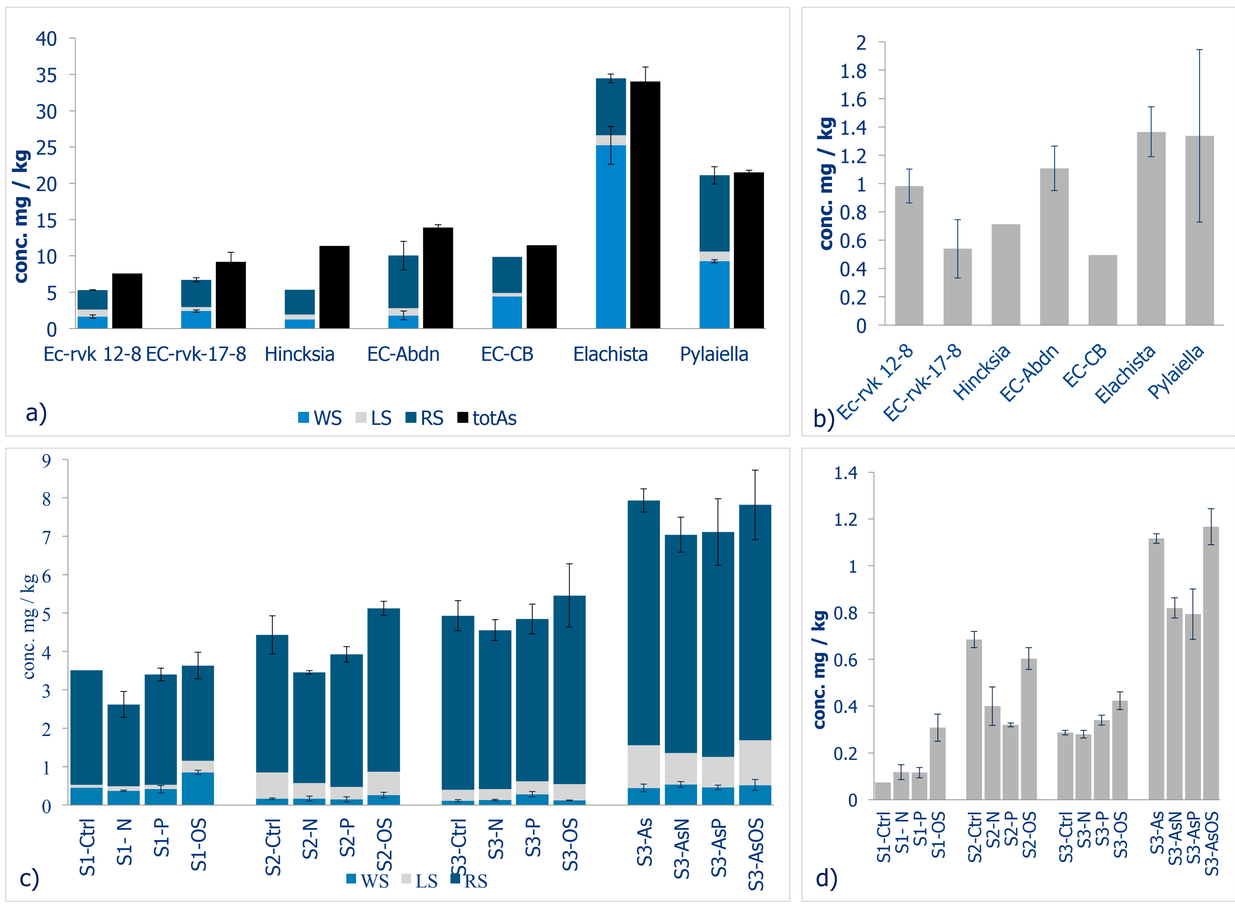

Figure 5. Distribution between water soluble arsenic species (WS), lipid soluble arsenicals (LS) and the arsenic still bound in the residue (RS) after sequential extraction for: a) Ectocarpales species found in natural habitat, includes the total arsenic (totAs) c) Ectocarpus cultures. Also a close up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

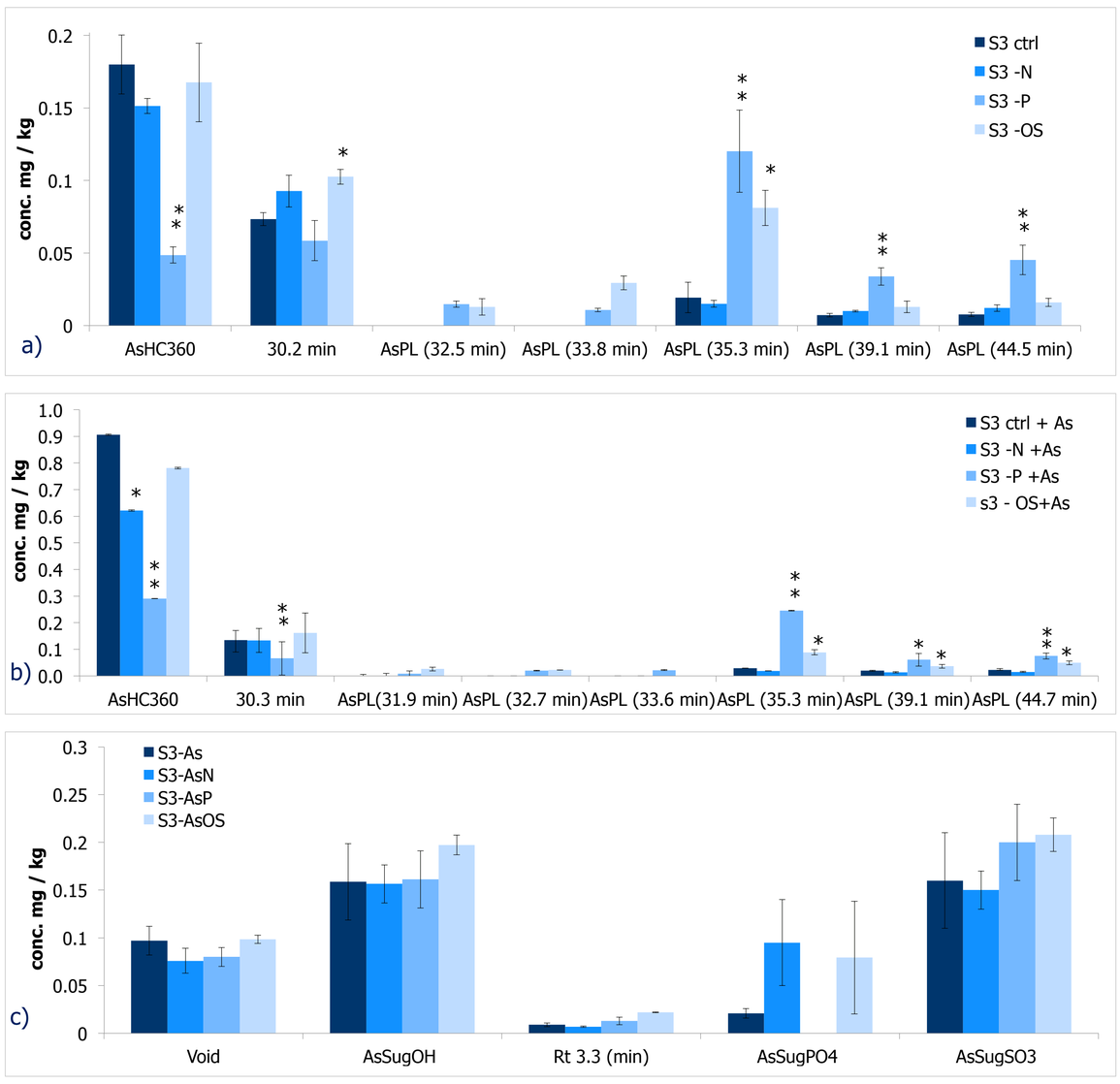

Figure 7. a) Arsenolipid profiles and quantification for S3. Condition statistically significant from ctrl using ANOVA: *P<0.05, **P<0.001 b) Arsenolipid profiles and quantification for S3 at enriched arsenic conditions. c) AsSugar profile and quantification for S3 + As.

Ásta H. Pétursdóttir (astap@matis.is; http://www.matis.is)
[1] Matís ltd [2]Dept of Chemistry, University of Aberdeen, Scotland. [3]Ocean lab, University of Aberdeen, Newburgh, Scotland.

rannís The Icelandic Research Fund 130235-052

Matís ohf. • Vínlandsleið 12 • 113 Reykjavík • Iceland • Tel +354 422 5000 • Fax +354 422 5001 • matis@matis.is • www.matis.is