Programmübersicht

Willkommen zur DGMS 2017

Application of LC-MS technique for intra-thallus profiling of polyphenolics in three species of fucoid algae

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Keywords: phlorotannins, brown algae, LC-MS

Introduction

Brown algae possess a unique group of polyphenolic secondary metabolites – phlorotannins, which apparently contribute to multiple physiological functions like herbivore deterrence, UV protection, cell wall formation and bioadhesion. These compounds are acetate-malonate derived polymers of phloroglucinol with different degree of polymerization leading to a wide range of molecular sizes (126 Da to 650 kDa). Depending on the nature of the linkages binding the polymers and on the number of hydroxyls, six major classes of phlorotannins have been defined [1]. A diversity of phlorotannin molecules and their ability to form multiple isomeric structures hamper their detailed analysis. Here we study phlorotannin profiles in different thallus zones of algae to elucidate the connection between allocation and physiological functions of different phlorotannin species.

Experimental section

Three representatives of the order Fucales (*Fucus vesiculosus*, *F. serratus* and *Pelvetia canaliculata*) were used as objects. Samples were taken from four thallus zones of each alga – thallus base, middle zone, vegetative apices and mature receptacles. Plant material was ground and extracted five times with 70% acetone. The acetone was evaporated and remaining water extract was purified via liquid-liquid separation with dichloromethane and then with ethyl acetate [2]. Chromatography was performed on Agilent 1100 instrument equipped with a C18 column (Gemini). MS data were acquired on Bruker Esquire 3000 Plus ESI ion trap mass spectrometer.

Results and discussion

The total amount of soluble phlorotannins varied from 5 to 24% DW and did not differ significantly between three algal species. Receptacles contained approx. 2-fold lower level of polyphenolics than vegetative tissues. Maximum phlorotannin content was found in the middle zone of the thallus for *F. vesiculosus* and in the vegetative tips - for the other two species. All the samples contained phlorotannins with the degree of polymerization (DP) varying from 3 to 50, presumably belonging to the classes of fucols and fucophlorethols. Mass spectrometry data allowed identification of more than 70 phlorotannins. The low molecular weight phenolics dominated in *F. vesiculosus*, and *P. canaliculata* had the maximum relative content of larger molecules (DP>20), which is consistent with literature data [4]. In all three algal species the small-sized phlorotannins (DP 3-8) were concentrated in the vegetative apices, where they presumably act as antioxidants and UV-protectors. The higher molecular weight polyphenols were more specific for middle and basal thallus zones where they might contribute to antifouling

activity and cell wall rigidification. Such a profile of phlorotannin distribution was especially prominent for the subtidal species *F. serratus*.

The research was supported by the Russian Foundation for Basic Research (project no. 17-04-01331)

Novel aspects

Fucus serratus and two more fucoid species accumulate low molecular weight phlorotannins in the vegetative apices.

References:

[1] Ragan M., Glombitza K. (1986) Progr Phycol Res 4: 130–241.

[2] Audibert L., Fauchon M., Blanc N., Hauchard D., Ar Galla E. (2010) Phytochem Anal 21: 399–405.

[3] Steevensz A., MacKinnon S., Hankinson R., Craft C., Connan S., Stengel D., Melanson J. (2012) Phytochem Anal 23: 547–553.

Application of LC-MS technique for intra-thallus profiling of polyphenolics in three species of fucoid algae

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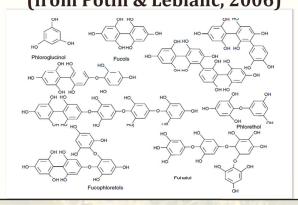
Introduction

Brown algae possess a unique group of polyphenolic secondary metabolites – phlorotannins, which apparently contribute to multiple physiological functions like herbivore deterrence, UV protection, cell wall formation and bioadhesion. These compounds are acetate-malonate derived polymers of phloroglucinol (Fig. 1) with different degree of polymerization leading to a wide range of molecular sizes (126 Da to 650 kDa). Depending on the nature of the linkages binding the polymers and on the number of hydroxyls, several classes of phlorotannins have been defined (Ragan, Glombitza, 1986). A diversity of phlorotannin molecules and their ability to form multiple isomeric structures hamper their detailed analysis. Here we study the total amount of polyphenolics as well as phlorotannin profiles in different thallus zones of algae to elucidate the connection between allocation and physiological functions of phlorotannin species with different degree of polymerization (DP).

Fig. 1. Representatives of different phlorotannin classes (from Potin & Leblanc, 2006)



Materials and Methods

Three intertidal species of the order Fucales differing in their ecology (*Fucus vesiculosus, F. serratus and Pelvetia canaliculata*) were used as objects. Samples were taken from four thallus zones of each alga – thallus base (stipe), middle zone, vegetative apices and mature receptacles (Figs. 2-3). Samples were taken from four thallus zones of each alga – thallus base (stipe), middle zone, vegetative apices and mature receptacles (Fig. 3). Plant material was ground and extracted five times with 70% acetone. The acetone was evaporated and remaining water extract was purified via liquid-liquid separation with dichloromethane and then with ethyl acetate (Audibert et al., 2010). Total amount of polyphenolics was determined with Folin-Ciocalteu assay. Chromatography was performed on Agilent 1100 HPLC equipped with a Gemini C18 column 150 x 2 mm, 5 µ (Phenomenex). MS data were acquired on Bruker Esquire 3000+ ESI ion trap mass spectrometer in negative mode (scan range: m/z 300-3000, target m/z 1000).

Fig. 2. Location of the thallus zones taken into analysis

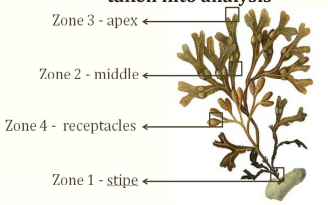


Fig. 3. Objects of the study – intertidal fucoid algae

Size - 70-130 cm, inhabiting subtidal and low intertidal zone

Size - 40-80 cm, inhabiting mid-intertidal zone

Size - 3-15 cm inhabiting high intertidal zone

Fig. 4. Total amount of the soluble phlorotannins in fucoid algae

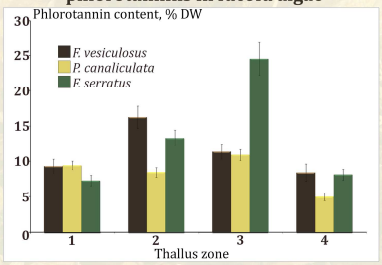
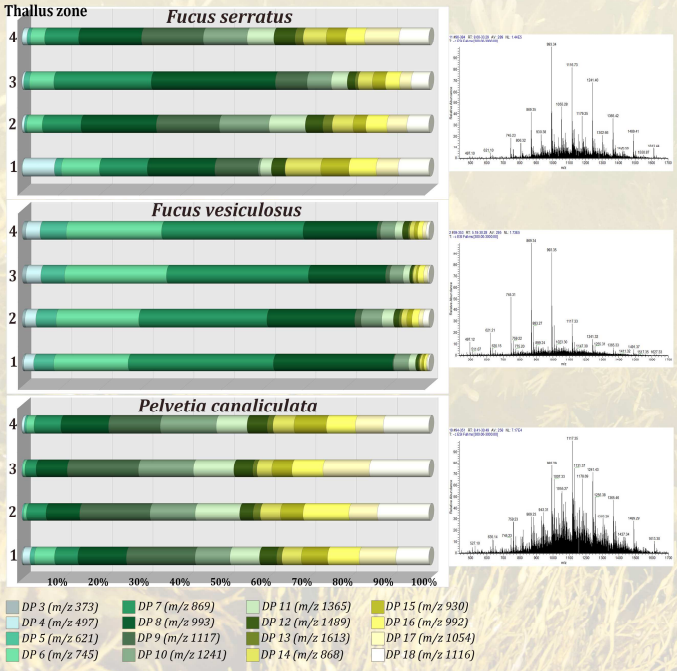


Fig. 5. Distribution of phlorotannins with different DP along the thalli of three fucoid algae and corresponding mass-spectra of major phlorotannin species



Results

The total amount of soluble phlorotannins varied from 5 to 24% DW and did not differ significantly between three algal species (Fig. 4). Receptacles generally contained lower level of polyphenolics than vegetative tissues. Maximum phlorotannin content was found in the middle zone of the thallus for *F. vesiculosus* and in the vegetative tips - for the other two species.

All the samples contained phlorotannins with the DP varying from 3 to 50, presumably belonging to the classes of fucols and fucophlorethols. Mass spectrometry data allowed identification of more than 70 phlorotannins. The low molecular weight phenolics dominated in *F. vesiculosus*, and *P. canaliculata* had the maximum relative content of larger molecules with DP>15 (Fig. 5), which is consistent with literature data (Steevensz et al., 2012).

The largest and most anatomically differentiated species (*F. serratus*) demonstrated the specific profile of phlorotannin distribution: the small-sized phlorotannins (DP 4-8) were mostly concentrated in the vegetative apices, where they presumably act as antioxidants and UV-protectors. The higher molecular weight polyphenols were more specific for middle and basal thallus zones where they might contribute to antifouling activity and cell wall rigidification.

Conclusions

Fucus serratus accumulates phlorotannins with DP 4-8 in the vegetative apices and higher molecular weight phlorotannins – in the thallus base. Such a distribution may reflect the specific functions of different phlorotannin groups.