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Comprehensive quantification and genome survey reveal the presence of novel phytohormone action modes in red seaweeds

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Abstract

Emerging work has suggested the existence of phytohormones in seaweeds, although chemical species, endogenous biosynthetic pathways, and signal transduction machineries remain poorly understood. We performed profiling of nine phytohormones with liquid chromatography-mass spectrometry and *in silico* genome-wide homology search to identify genes involved in biosynthesis and signal transduction of hormones in red algae. It was demonstrated that two Bangiophycean algae, *Bangia fuscopurpurea* and *Pyropia yezoensis,* possessed indoleacetic acid (IAA), N^6-(Δ^2-isopentenyl)adenine (iP), abscisic acid (ABA) and salicylic acid, although *trans*-zeatin, dihydrozeatin, gibberellinA$_1$ and A$_4$, and jasmonate were not detected. Results of genome-wide survey demonstrated that Bangiophycean algae produce iP and ABA *via* pathways similar to those in terrestrial plants. However, these seaweeds lack homologues of already known factors participating in perception and signal transduction of IAA, iP, and ABA, indicating that the action modes of these phytohormones in red seaweeds differ from those elucidated in terrestrial plants. These findings shed lights on evolutional divergence of signal transduction pathways of phytohormones in plants.

Key words: *Bangia fuscopurpurea*, liquid chromatography-tandem mass spectrometry, phytohormone, *Pyropia yezoensis*, quantitative profiling, comparative genomics

Introduction

Substantial previous work has identified ten classes of plant hormones: auxin, cytokinins, abscisic acid (ABA), gibberellins (GAs), salicylic acid (SA), jasmonates (JAs), brassinosteroids, strigolactones, nitric oxide, and peptide hormones, and the biological functions of these phytohormones in growth, development, and environmental stress response have been extensively studied (Weyers and Paterson 2001; Vanstraelen and Benková 2012). The biosynthetic pathways and action modes of phytohormones have been studied mainly in the green lineage, where the functional involvement of these hormones in various physiological aspects has been analyzed based on genome-wide comparisons of genes involved in hormone biosynthesis and actions (Johri 2008; Lau et al. 2009; Takezawa et al. 2011; De Smet et al. 2011; Yue et al. 2014; Wang et al. 2015). Of marine plants, brown seaweeds are on the frontiers of phytohormone research. For instance, auxin functions in early embryo development and regulating the branching pattern of sporophytes (Basu et al. 2002; Le Bail et al. 2010), and comparative genomic surveys have identified factors involved in biosynthesis and signal transduction of phytohormones (Le Bail et al. 2010; Kieseleva et al. 2012).

Little is known about the presence and physiological functions of hormones in red seaweeds to date. Primary photosynthetic eukaryotes like red alga and green plants (including green algae and land plants) belong to the Archaeplastida, whereas secondary photosynthetic eukaryotes like brown seaweeds belong to the Heterokonta (also called Stramenopiles), which diverged by the secondary endosymbiosis of an ancestral red alga (Le Corguillé et al. 2009; Nozaki et al. 2009; Dorrell and Smith 2011). Red algae therefore are supposed to possess ancestral features of photosynthetic eukaryotes. Accordingly, identification and quantitative measurement of plant hormones in red seaweeds will provide fundamentally valuable information to help our understanding of the evolutionary origin and function of phytohormones in the Archaeplastida.

Phytohormones consist of structurally and characteristically unrelated small compounds. Since they usually exist only in trace amounts in plants, separation and quantification of phytohormones can be technically difficult. Biological chemists have therefore developed high-sensitivity methods for comprehensive analysis of plant hormones, such as liquid chromatography-ESI-tandem mass spectroscopy (LC-ESI-MS/MS) (Izumi et al. 2009; Kojima et al. 2009; Kanno et al. 2010; Tokuda et al. 2013) that successfully provided new information about quantitative changes in plant hormone contents during the development of terrestrial plants (Kanno et al. 2010; Tokuda et al. 2013).

Presence of phytohormones and their significance in seaweeds has been implied. Historically, seaweed extract-containing manures have widely been employed in industrial applications to promote plant growth (see Khan et al. 2009; Craigie 2011), and analysis of the active ingredients in these manures identified phytohormones, i.e. auxin, cytokinins, ABA, and GAs (Sanderson et al. 1987; Tay et al. 1985; Strik et al. 2014). Conversely, the functional significance of phytohormones in seaweed growth has been examined by exogenous application to seaweeds (for instance, Davidson 1950; Yokoya and Handro 1996; Lin and Stekoll 2007); however, these types of experiments have not provided direct and sufficient

evidence to ascertain the presence and endogenous production of plant hormones in seaweeds *in vivo*. Many efforts have been made to detect and quantify phytohormones in seaweeds by applying methods established for terrestrial plants, such as gas chromatography-mass spectrogram GC-MS, LC-MS, and high-performance liquid chromatography-MS (Jacobs et al. 1985; Sanderson et al. 1987; Zhang et al. 1993; Ashen et al. 1999; Stirk et al. 2004; Stirk et al. 2009; Gupta et al. 2011). Recently, GC-tandem MS (GC-MS/MS) and LC-MS/MS have been used for simultaneous quantification of plant hormones in red seaweeds (Yokoya et al. 2010; Wang et al. 2014). These technical improvements enabled sensitive and high-throughput analysis of plant hormones in seaweeds. These analyses suggested the presence of phytohormones such as auxin, cytokinins, ABA and ethylene in various kinds of seaweeds. However, the pathways for phytohormone biosynthesis in seaweeds have not been fully addressed by survey for genes encoding enzymes involved in phytohormone biosynthesis, except for auxin biosynthetic pathways in the brown seaweed *Ectocarpus siliculosus* (Le Bail et al. 2010).

Toward functional analysis of plant hormones in red seaweeds, we here improved a simultaneous profiling method for plant hormones using LC-ESI-MS/MS in the Bangiophycean algae, *Pyropia yezoensis* and *Bangia fuscopurpurea*, that arose very early in evolution of Plantae. In addition, since molecular biological bases of metabolism and response of phytohormones in red seaweeds are still missing, we performed extensive genome-wide surveys for genes involved in biosynthesis and signal transduction of plant hormones using the genome sequence of *P. yezoensis* (Nakamura et al. 2013) and expression sequence tag (EST) information for *Porphyra umbilicalis* and *Porphyra purpurea* (Chan et al. 2012a; Chan et al. 2012b; Stiller et al. 2012). Our results clearly indicated the presence of plant hormones in Bangiophycean algae; however, the genome-wide survey revealed likely differences in action modes of plant hormones between red seaweeds and terrestrial plants.

Materials and methods

Algal strains and culture conditions

Gametophytes and sporophytes of culture strains of *P. yezoensis* strain U51 were kindly provided by the Marine Resources Research Center of Aichi Fisheries Research Institute and gametophytes of *B. fuscopurpurea* were originally collected at Esashi, Hokkaido, Japan on May 14, 2010 (Hirata et al. 2011). These strains were grown in ESL medium (Kitade et al. 2002) at 15°C under irradiation of 70 μmol m^{-2} s^{-1} provided by cool white fluorescent lamps with a photo-period of 10 h light:14 h dark. The medium was bubbled continuously with filter-sterilized air and changed weekly.

Quantification of phytohormones

Fresh laboratory-cultured seaweed was harvested, lyophilized, and ground in a mortar in liquid nitrogen. Approximately 50 mg (dry weight) of precisely weighed algal body was used for each extraction, unless

otherwise indicated. Extraction solvent mixture (4 mL) consisted of 80% (v/v) acetonitrile and 1% (v/v) acetic acid containing internal standards as described below was added into the mortar to suspend powdered samples. Viscosity of the buffer increased transiently and then substantially decreased during incubation at 4°C for 1h. After a centrifugation at 3000 g for 10 min at 4°C, the supernatant of extracts were collected. The pellet was rinsed with 80% acetonitrile containing 1% acetic acid, followed by a centrifugation at 3000 g for 10 min at 4°C. Supernatants were merged and successively pre-treated as described by Tokuda et al. (2013). In brief, the supernatants were evaporated to water containing 1% acetic acid and applied to Oasis HLB 1cc (30 mg) extraction cartridge (Waters Corporation, Milford , MA, USA) equilibrated with 1% acetic acid. After washing with 1 mL of 1% acetic acid, hormones were eluted with 2 mL of 80% acetonitrile containing 1% acetic acid. Acetonitrile in the eluate was evaporated with centrifugal evaporator to remain water, acidified with 1% acetic acid and then applied to Oasis MCX 1cc extraction cartridge (30 mg, Waters) equilibrated with 1% acetic acid. After washing the cartridge with 1 mL of 1% acetic acid, the acidic fraction containing GA_1, GA_4, IAA, ABA, JA, A-Ile and SA was eluted with 2 mL of 80% acetonitrile containing 1% acetic acid. Two hundred microliters of the fraction were evaporated to dryness and reconstituted in 50 µL of 1% acetic acid for analysis of SA. The MCX cartridge was successively washed with 1 mL of 5% (v/v) ammonia in water, and basic fraction containing tZ, DHZ and iP was eluted with 40% acetonitrile containing 5% ammonia. The basic fraction was evaporated to dryness and reconstituted in 1% acetic acid for analysis of tZ, DHZ and iP. The acidic fraction was further applied to Oasis WAX 1 cc (30 mg) extraction cartridge (Waters) equilibrated with 1% acetic acid following to evaporation of acetonitrile. After washing with 1 mL of 1% acetic acid, GA_1, GA_4, IAA, ABA, JA and JA-Ile were eluted with 2 mL of 80% acetonitrile containing 1 % acetic acid. The eluates were evaporated to dryness and reconstituted in 1% acetic acid and subjected to analysis of GA_1, GA_4, IAA, ABA, JA and JA-Ile. Stable isotope-labeled compounds used as internal standards were: D_2-IAA, D_7-IAA (CDN Isotopes, Canada); D_2-GA1, D_2-GA4, D_6-iP, D_5-tZ, D_3-DHZ, D_6-ABA, D_4-SA (OlChemim, Czech Republic), D_2-JA (Tokyo Kasei, Japan); and $^{13}C_6$-JA-Ile (kindly gifted by Dr. Yusuke Jikumaru, Riken, Japan; present affiliation: Agilent Technologies Japan, Ltd). The LC methods are detailed in Table S1. GA_1, GA_4, IAA, ABA, JA and JA-Ile were examined with LC method No. 1, while LC method No. 2 was applied for tZ, DHZ and iP. SA was analyzed using LC method No. 3. Detailed information of internal standards is summarized in Table S2. Hormones were analyzed with an LC-electrospray ionization (ESI)-MS/MS system (Agilent1260-6410) equipped with a ZOBRAX Eclipse XDB-C18 column and XDB-C8 Guard column, and peak areas were determined using MassHunter Workstation software (ver. B.04.00, Agilent Technologies Inc.).

Identification of putative genes involved in biosynthesis and signal transduction of plant hormones

Genes of *Arabidopsis thaliana* and *Cyanidioschyzon merolae* involved in biosynthesis and signal transduction of plant hormones were used as queries for homology searching via BLAST in the web sites for *P. yezoensis* ESTs (http://est.kazusa.or.jp/en/plant/porphyra/EST/) and ESTs of *P. purpurea* and *P.*

umbilicalis (NiroBLAST: http://dbdata.rutgers.edu/nori/). In addition, *P. yezoensis* genome information from Nakamura et al. (2013) was also searched. Since contigs of ESTs encoding candidates from red seaweeds are usually not full-length, protein domain analysis and sequence alignment were also performed. Conserved domains of their products were identified by CD-search in the NCBI web site (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and amino acid sequences were aligned by CLUSTALW (http://www.genome.jp/tools/clustalw/) with homologues identified or annotated previously. These results were confirmed by reverse BLAST search performed with identified EST or contigs as queries (data not shown).

Results

Applicability of the method established in terrestrial plants for analyzing IAA contents in *P. yezoensis*

The first step of our plant hormone analysis in red seaweeds was to test the applicability of methods established for terrestrial plants. Thus, we applied the high-throughput and comprehensive analysis method using LC-ESI-MS/MS established by Tokuda et al. (2013) to examination of *P. yezoensis* sporophytes. This method can identify IAA, cytokinins [N^6-(Δ^2-isopentenyl)adenine, iP; *trans*-zeatin, tZ; ABA, gibberellins (GA1 and GA4), JA, JA-Ile, and SA from a specimen with internal standards, D_2-IAA, D_6-iP, D_5-tZ, D_6-ABA, D_2-GA1, D_2-GA4, D_2-JA, $^{13}C_6$-JA-Ile, and D_6-SA. Additionally, we utilize D_3-dihydrozeatin (DHZ) as the internal standard for DHZ in this study.

  The presence of IAA, iP, ABA and SA was confirmed, whereas tZ, gibberellins and JAs were not detected (Fig. 1). DHZ also was not detectable (data not shown). Although tiny peaks for tZ and JA were observed in chromatograms, these peaks did not reflect the presence of endogenous tZ and JA in the sporophytes but rather contamination of unlabeled compounds in the internal standards. The proportion of contamination of unlabeled compounds in D_2-JA and D_5-tZ were determined to be 1.38% and 0.023%, respectively, by LC-MS/MS analysis (data not shown), and the areas of these tiny peaks were comparable to the amount of unlabeled contaminants (Fig. 1). Areas in elution profiles showing ABA and SA peaks were not reproducible in independent examinations, and their trace amounts prevented quantitative detection. Based on these results, we proposed that the method by Tokuda et al. (2013) is applicable for analysis of IAA and iP in sporophytes of *P. yezoensis*, although it is necessary to improve methods for quantitative determination of ABA and SA.

Unexpected presence of an intracellular factor preventing IAA profiling

We observed that areas of IAA and D_2-IAA in chromatograms increased in parallel with the changes in volume of *P. yezoensis* sporophytes (Fig. 2a), whereas areas of D_6-iP were roughly equal despite variation of the volume of starting material (Fig. 2b). We initially suspected that the efficacy of extraction solvent

was suboptimum for IAA for some reason. Therefore, we tried modifying the extraction method to improve recovery and achieve an optimum extraction, in case the imperfect extraction was the underlying reason for the non-linear quantification compared with the amount of the starting material. Phytohormones were successively extracted from ground samples in 80% acetonitrile containing 1% acetic acid, 80% methanol containing 1% acetic acid, 80% acetone containing 1% acetic acid, and chloroform/methanol (1:1) containing 1% acetic acid. Each fraction was examined by LC-MS/MS. The multiple reaction monitoring (MRM) chromatogram of mass-to-charge ratio (m/z) of 176>130 corresponding to IAA was mostly extracted at the first extraction step (80% acetonitrile containing 1% acetic acid) and fully extracted by the second step (80% methanol containing 1% acetic acid, Fig. S1 lower). However, the MRM chromatogram of m/z 178>132 was detected in all extraction steps (Fig. S1 upper), indicating the presence of an unidentified substance whose MRM transition matched and retention time was similar with those of D_2-IAA. This unidentified substance showed completely different extractability compared to IAA. These findings led us to assume that detection of D_2-IAA was hampered by an endogenous factor(s) whose amounts increased in parallel with increases in weight of algal samples.

To test the possibility that the D_2-IAA internal control prevents quantitative detection of endogenous IAA, the acetonitrile extracts were subjected to solid phase extraction and LC-MS/MS analysis without addition of D_2-IAA. When m/z 176>130 was monitored, which is the most abundant ion, endogenous IAA was clearly detected (Fig. 3a). When the MRM to monitor D_2-IAA (m/z 178>132) was used, a large peak with a retention time that overlapped with that of the peak for D_2-IAA was observed even without an addition of D_2-IAA (Fig. 3b). A substance derived from cellular extracts of *P. yezoensis* apparently prevented quantitative analysis of D_2-IAA. Therefore, we concluded that quantification of IAA utilizing D_2-IAA as the internal standard is not feasible in *P. yezoensis*, indicating the limitation in application of the method of Tokuda et al. (2013) to this seaweed.

To avoid the effect of this unidentified substance, which was found at m/z 178>132, we instead analyzed a minor fragment ion derived from IAA and D_2-IAA (MRM: m/z 176>103 and 178>105, respectively; fragmentor voltage: 90 V, collision energy: 34 V, ESI mode: positive). As a result, we successfully avoided the substance that hampered quantification of the D_2-IAA and endogenous IAA; however, the sensitivity was reduced ca 10-fold (data not shown). Thus, this method was not effective for quantification of the trace amounts of IAA in *P. yezoensis*.

We next tested two other commercially available isotope-labeled internal standards, D_5-IAA and D_7-IAA. We first monitored for substances that might hamper the analysis in algal extracts without addition of internal standard. As shown in Fig. 3c, we observed an endogenous substance of which retention time overlapped with IAA and thus it was deduced to interfere the detection of D_5-IAA (compare Figs 3a and 3c). By contrast, when m/z 183>137 was monitored, which is for D_7-IAA, no peak overlapping with endogenous IAA was observed (Fig. 3d). Fragment ions derived from the purchased D_7-IAA were further analyzed to ensure their suitability as the internal standards. As shown in Fig. S2, three fragment ions derived from D_7-IAA were detected (m/z 183>137, 136, 135) and none of these were

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supposedly interfered with substances in the algal extracts. Using D_7-IAA, therefore, we can accurately quantify endogenous IAA.

Taken together, these results indicate that the sensitive and comprehensive analysis method for IAA, iP, tZ, gibberellins and JAs was successfully developed for sporophytes of *P. yezoensis*.

Applicability of the new method to gametophytes of Bangiophyceae

We next tested whether our method was applicable to gametophytes of *P. yezoensis* in addition to sporophytes mentioned above. Preparation of algal extracts and separation of plant hormones were performed according to our method for sporophytes. IAA, iP, ABA, and SA were present, whereas tZ, DHZ, gibberellins, and JAs were not detected (Table 1). In addition, quantitative detection of ABA and SA was similarly prevented in gametophytes. These results clearly demonstrated that in *P. yezoensis*, plant hormone composition of gametophytes was essentially the same as that in sporophytes. When gametophytes of *B. fuscopurpurea* were used as starting materials, the similar results were obtained (Table 1). Thus, the method established for the sporophyte of *P. yezoensis* is applicable to gametophytes of Bangiophycean algae.

The results of quantification of phytohormones in *P. yezoensis* and *B. fuscopurpurea* are summarized in Table 1. IAA content was very high in both gametophytes and sporophytes of *P. yezoensis* compared to gametophytes of *B. fuscopurpurea*. These findings suggest a functional significance of IAA in gametophyte and sporophyte generations in *P. yezoensis*, although it is necessary to confirm this possibility by biological approaches.

Genome-wide survey of genes involved in biosynthesis and signal transduction of plant hormones in red seaweeds

The presence of IAA, iP, ABA, and SA led us to consider whether red seaweed genomes have homologues of genes involved in biosynthetic pathways and signal transduction systems of these plant hormones in other species. Accordingly, we performed genome-wide surveys for genes potentially involved in these processes in Bangiophysiae species for which nuclear genomes have been sequenced or large-scale EST information has been produced (Chan et al. 2012a; Chan et al. 2012b; Nakamura et al. 2013; Stiller et al. 2012). We identified some genes predicted to be involved in biosynthesis of iP and ABA (Table S3), although there was no homologue of IAA and SA biosynthetic genes. Remarkably, we found no genes encoding proteins similar to known factors involved in signal transduction of IAA, iP, ABA, and SA.

We identified ESTs encoding a cytokinin riboside 5'-monophosphate phosphoribohydrolase (LOG) homologue in *P. purpurea* and *P. unbilicalis* (Fig. S3). LOG catalyzes the final step of cytokinin biosynthesis by conversion of iP nucleotide and tZ nucleotide to cytokinin molecules (Sakakibara, 2006).

We could not find a homologue of cytochrome P450 mono-oxygenase (CYP735A), which is involved in production of tZ nucleotide from iP nucleotide (data not shown).

The precursors of ABA are carotenoids such as violaxanthin and neoxanthin (Mikami and Hosokawa 2013; Nambara and Marion-Poll 2005). However, the major carotenoid in red seaweeds is zeaxanthin, and whether violaxanthin and neoxanthin are produced from zeaxanthin in red seaweeds is unknown (Mikami and Hosokawa 2013). The presence of ABA was therefore unexpected, although endogenous ABA has been already detected in various kinds of red seaweeds (Yokoya et al. 2010; Wang et al. 2014). Surprisingly, we found genes encoding zeaxanthin epimerase predicted to catalyze production of violaxanthin from zeaxanthin in *P. yezoensis* as well as *P. purpurea* and *P. umbilicalis* (Figs. 4 and S4). In addition, genes encoding 9-cis-epoxycarotenoid dioxygenase (NCED), xanthoxin dehydrogenase (XanDH, also known as ABA2) and abscisic aldehyde oxidase (AAO3) involved in ABA biosynthesis from carotenoids (Nambara and Marion-Poll 2005) were found in *P. purpurea* and *P. umbilicalis* (Figs. 4 and S5-S7). Moreover, a XanDH homologue was also found in *P. yezoensis* (Figs. 4 and S6). These findings strongly support the presence of an ABA biosynthetic pathway in Bangiophycean algae, which is consistent with our detection of ABA in red seaweed (Fig. 1).

Discussion

In this work, the system for highly-sensitive and simultaneous profiling of plant hormones by LC-ESI-MS/MS established for territorial plants was modified and adapted for analysis of plant hormones in red seaweeds by optimization of stable isotope-labeled internal standards. This modified method was fully applicable to *P. yezoensis* and *B. fuscopurpurea*, and identified IAA, iP, ABA, and SA in these algae as conserved plant hormones. Moreover, identification of genes homologous to known genes involved in biosynthesis of iP and ABA in other plants supports the profiling results.

Among the methodological modifications, employing D_7-IAA as the internal control was a key element to quantify IAA in red seaweeds. Our results revealed the unsuitability of D_2-IAA and D_5-IAA as internal standards, because they may lead to underestimation of IAA contents, due to an unidentified cellular factor showing a similar ion signal to those potential internal controls. In the previous reports, $[^2H_5]IAA$ (Stirk et al. 2009) and $^{15}N/^2H_5$-IAA (Yokoya et al. 2010) were used as the internal controls in GC-SIM-MS and LC-MS/MS, respectively. Use of $[^2H_5]IAA$ (identical to D_5-IAA) is deduced to confront the same problem of interference in detection. In addition, $^{15}N/^2H_5$-IAA should have the MRM transition 182>136 m/z. Our results highlight a need to reconfirm the quantification of IAA contents showed in those reports using D_7-IAA as the internal standard.

A LC-MS/MS approach somewhat similar to ours was recently reported for high-throughput analysis of hormones in the red seaweed *Pyropia haitanensis* (Wang et al. 2014). In this report, they utilized external standards instead of internal standards for quantification. An external standard method has intrinsic drawbacks, such as extraction efficiency and ionization efficiency between materials. The internal standard method yields better precision and more accurate quantification data than an external

standard method.

Although quantitative detection of ABA was not successful in the present study, the finding of a complete set of genes required for production of ABA from zeaxanthin (Fig. 4) strongly supports the presence of ABA in Bangiophyceae. ABA may be biosynthesized only under a certain environmental conditions, a factor that we did not test in this study. Despite the presence of enzymes involved in ABA biosynthesis, there were no homologues for ABA receptors, PYR/PYL/RCAR proteins (Cutler et al. 2010; Klingler et al. 2010) (data not shown), suggesting the presence of an unknown mode of ABA action in red seaweeds. In fact, it is generally thought that the carotenoid biosynthetic pathway stops at zeaxanthin in red seaweeds (Schubert et al. 2006; Esteban et al. 2009; for review, Mikami and Hosokawa 2013). Therefore, confirmation of the biological significance of the putative ABA biosynthetic genes found in the present study will require chemical identification of violaxanthin and neoxanthin in red seaweeds and functional characterization of these genes.

It is worth noting that we did not find any gene for violaxanthin de-epoxidase (VED) in red seaweeds as described by Yang et al. (2014) in *P. umbilicalis*. Thus, it remains an outstanding question whether Bangiophyceae has the violaxanthin cycle that is involved in the light-regulated switching of photosystem II from a light-harvesting state to an energy dissipating state (Jahns and Holzwarth 2012). Since the violaxanthin cycle is important to acclimate to light conditions, it is necessary to confirm the absence of VED for further understanding of properties of photosynthesis in Bangiophyceae.

Cytokinins like iP, tZ and DHZ are biosynthesized from dimethylallyl diphosphate (DMAPP) from the methylerythritol phosphate (MEP) pathway, whereas DMAPP derived from the mevalonate (MVA) pathway is catalyzed to *cis*-zeatin (cZ) (Sakakibara 2006). The MEP pathway-derived DMAPP is converted to iP nucleotide with adenine phosphate-isopentenyltransferase (IPT) and then to tZ nucleotide with CYP735A, following a conversion of iP and tZ nucleotides to cytokinins with LOG (Sakakibara, 2006). Despite the presence of LOG (Fig. S3), neither CYP735A nor IPT homologues were found in Bangiophyceae (data not shown), suggesting a possibility that iP is biosynthesized *in vivo* as a unique cytokinin in Bangiophysiae. Although it would be consistent with our results showing the absence of tZ- and DHZ-type cytokinins (Figs. 1 and 2, Table 1), further survey is necessary to confirm the presence of tZ and DHZ depending on the MEP pathway. In fact, LOG is also involved in biosynthesis of cZ-type cytokinin in the MVA pathway (Sakakibara 2006). Since cZ was not analyzed in the present study, the presence of cZ and the MVA pathway for cytokinin biosynthesis should be confirmed in Bangiophycean algae. The unique presence of iP in Bangiophyceae (Fig. 1 and Table 1) is inconsistent with the previous reports showing the presence of isopentel (iP and tZ) and aromatic cytokinins in various kinds of red seaweeds (Zhang et al. 1993; Stirk et al. 2003). Further analysis of endogenous cytokinins and genes related to cytokinin biosynthesis are therefore necessary. Moreover, since iP has a weak effect as cytokinin in terrestrial plants (van Staden and Drewes 1991), it will be interesting to elucidate why Bangiophyceae have only iP as cytokinin and how iP acts as a regulator of cellular processes.

In terrestrial plants, cytokinins are perceived by the histidine kinases AHK2, AHK3 and CRE1/AHK4 (To and Kieber 2008). It is clear that red seaweed nuclear genomes have no genes encoding

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these histidine kinases and thus the action mode of cytokinin is proposed to differ in red seaweeds and in terrestrial plants, suggesting differences in receptor and cytokinin-dependent gene expression systems. In fact, plastid genomes of Bangiophyceae carry a gene encoding the histidine kinase Ycf26 (Puthiyaveetil and Allen 2009; Tanaka 2011). It has been established that in the cyanobacterium *Synechocyctis* sp. 6803, a Ycf26 designated Hik33 is involved in the perception of cold and hyperosmotic stresses (Mikami et al. 2002; Mikami and Murata 2003). Thus, there is a possibility that plastidial Ycf26 of red seaweeds also plays a role in environmental stress perception; however, it remains unknown whether it functions as a cytokinin receptor.

Another obvious finding is the lack of gene homologues encoding auxin signal transduction components such as TIR1, ABP1, IBR5, Aux/IAA and ARF and auxin transporters PIN and AUX1 in Bangiophyceae (data not shown), which is consistent with observations in the brown seaweed *E. siliculosus* (Le Bail et al. 2010), the unicellular red alga *Cyanidioschyzon merolae*, and the green algae *C. reinhardtii* and *Volvox carteri* (Lau et al. 2009; De Smet et al. 2011). Therefore, algae seem to have novel molecular mechanisms for perception and intracellular signaling of IAA, different from the signal transduction systems elucidated in terrestrial plants. Confirmation of these possibilities could provide new insights into the regulation of physiological processes by IAA in plants.

In summary, our study identifies IAA, iP, ABA, and SA as conserved plant hormones in red seaweeds with improved comprehensive analytical methods. In addition, it is clear that seaweeds largely lack factors involved in signal transduction of plant hormones. These findings unambiguously demonstrate endogenous production of these hormones and also strongly suggest the presence of novel systems for activation of plant hormone-dependent physiological regulations in seaweeds. Therefore, quantitative profiling of plant hormones in development and environmental stress responses and approaches to identify molecules involved in signal transduction of IAA, iP and ABA in Bangiophyceae through genomic studies are highly important to understand origin and diversity of modes of action of plant hormones.

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Figure Legends

Fig. 1 Detection of phytohormones in sporophytes of *P. yezoensis* using LC-MS/MS. MRM chromatograms of each hormone (red, lower) are shown by comparison with those of corresponding internal standards (blue, upper). Abundance of hormones was examined with the appropriate selected ion at specific m/z for each hormone and internal standard as indicated in Supplementary Table S2. D_2-IAA was used as the internal standard for IAA. The upper number on chromatogram peaks indicates measured retention time for the peak, and lower number indicates calculated peak area ratio.

Fig. 2 Unsuitability of D_2-IAA as an internal standard for quantitative analysis of endogenous IAA. **(a)** Dependency of the increase of D_2-IAA and IAA (blue and red MRM chromatograms, respectively) on amounts of starting materials, which prevents quantitative analysis of IAA. Detection was performed by adjustment of MRM to 178>132 m/z and 176>130 m/z. **(b)** D_6-iP as a suitable internal standard for quantification of iP. Chromatogram areas of D_6-iP were roughly equal and did not depend on the starting volumes of materials. Detection was performed by adjustment of MRM to 210>137 m/z and 204>136 m/z.

Fig. 3 Avoiding the negative effects of unidentified cellular factors on IAA quantification by changing isotope-labeled IAA internal controls. **(b)** and **(c)** reveal the unsuitability of D_2-IAA and D_5-IAA as internal controls, because unidentified cellular factors were detected as peaks overlapping to that of endogenous IAA **(a)** when MRM was adjusted to 178>132 m/z for D_2-IAA and 181>135 m/z for D_5-IAA. By contrast, adjusting MRM to 183>137 m/z did not reveal any peak overlapping that of endogenous IAA **(d)**, demonstrating the usefulness of D_7-IAA as an internal control for measurement of IAA in *P. yezoensis*. The mass-to-charge ratios (m/z) are shown above each panel. The upper number on chromatogram peaks indicates measured retention time for the peak, and lower number indicates calculated peak area ratio.

Fig. 4 Conservation of ABA biosynthetic enzymes in Bangiophyceae. The ABA biosynthetic pathway is shown, with red seaweed species in which genes encoding corresponding enzymes were identified listed to right in red. Zeaxanthin epimerase (ZEP), 9-cis-epoxycarotenoid dioxygenase (NCED), xanthoxin dehydrogenase (XanDH) and abscisic aldehyde oxygenase (AAO3) homologues were identified in red seaweeds, suggesting the presence of an ABA biosynthetic pathway similar to that in terrestrial plants (Nambara and Marion-Poll, 2005) and thus endogenous ABA in these organisms. Since enzymes involved in production of *cis*-isomer of violaxanthin and neoxanthin are unknown, homology searches for these

16

enzymes were not performed. Biosynthesis of zeaxanthin in red seaweeds is described in Mikami and Hosokawa (2013).

Legends to electronic supplemental materials

Table S1 LC-ESI-MS/MS methods used in the present study

Table S2 Parameters of isotope-labeled internal controls for LC-ESI-MS/MS analysis

Table S3 Blast search results for cytokinin and ABA biosynthetic genes

Fig. S1 Presence of a cellular substance preventing quantitative analysis of IAA. Sequential stepwise extraction of plant hormones was performed to examine the efficiency of extraction procedure. Ground samples were treated with solvents in the order of 80% acetonitrile containing 1% acetic acid, 80% methanol containing 1% acetic acid, 80% acetone containing 1% acetic acid and chloroform/methanol (1:1) containing 1% acetic acid. Each extract was examined by LC-MS/MS, for which monitoring was done by a MRM chromatogram at178>132 m/z for IAA (upper) and 176>130 m/z for D_2-IAA (lower) and. IAA was completely extracted by the second step, whereas an unidentified cellular substance, which has an MRM transition overlapping that of D_2-IAA, appeared in all extraction steps.

Fig. S2 Confirmation of the utility of D_7-IAA as an internal standard. LC-MS/MS performed with MRM at 183>135, 183>136 and 183>137, resulting in detection of three corresponding fragment ions derived from D_7-IAA. As Figure 3 indicates the absence of any cellular substance with MRM transition at 183>137 m/z, D_7-IAA can be used as the internal standard for IAA quantification.

Fig. S3 LOG homologues in *P. purpurea*. Three ESTs, esGAQG33Y01DF64M, esGAQG33Y01DSNGI and esContig5176, were found by homology search at NoriBLAST and designated PpLOG1, PpLOG2, and PpLOG3, respectively. These clones are partial and thus full-length information is not yet obtained. In the upper part, amino acid sequences and CD-search results of LOG homologues obtained using the NCBI Conserved Domain Database (CDD; www.ncbi.nlm.nih.gov/cdd) are represented. In the lower part, amino acid alignment obtained by CLUSTALW is shown, by which it is clear that PpLOG1 and PpLOG2 correspond to the N-terminal part of LOG and a short PpLOG3 sequence aligns with the middle part of LOG. Plant LOGs are classified into clade I and clade II (Kuroha et al. 2009). AtLOG1, AtLOG4, and OsLOGL2 belong to clade I, whereas AtLOG8, OsLOGL1, and OsLOGL4 are members of clade II. Since EST information of *P. purpurea* homologues is partial, it is difficult to determine the clade to which they belong. Accession numbers: AtLOG1, Q8RUN2; AtLOG4, Q9LFH3; AtLOG8, Q84MC2; OsLOGL1,

Q8LR50; OsLOGL2, B9F166; OsLOGL4, Q851C7.

Fig. S4 Bangiophycean ZEP homologues. The cDNAs encoding ZEP homologues were identified in *P. yezoensis*, *P. purpurea* and *P. umbilicalis*. For *P. yezoensis* ZEP (PyZEP), the full-length information was obtained as contig19911_g4901 from the *P. yezoensis* genome information by searching with conserved amino acid sequence of ZEP. Two ESTs, esisotig06175 of *P. purpurea* and esisotig05144 of *P. umbilicalis* were found by homology searches at NoriBLAST with the ZEP sequence from *A. thaliana* (AtZEP) and designated PpZEP and PuZEP, respectively. Amino acid sequences and CD-search results of red algal ZEP homologues and AtZEP, which were obtained using the NCBI Conserved Domain Database (CDD; www.ncbi.nlm.nih.gov/cdd), are represented. In addition, amino acid alignment obtained by CLUSTALW is shown. According to the above information, it is clear that Bangiophycean ZEP homologues lack the Forkhead-associated (FHA) domain found at the C-terminal region of AtZEP, suggesting differences in regulatory mode in ZEP function between red seaweeds and terrestrial plants. Accession numbers: AtZEP, Q9FGC7; OsZEP from rice, Q0JCU7; ZmZEP from corn, ACG42893.

Fig. S5 Bangiophycean NCED homologues. By homology searches at NoriBLAST with NCED sequences from *A. thaliana* (NECD9; accession no. AEE36100) and the unicellular red alga *C. merolae* (CmNCED; CMS362C, accession no. XP_005538977) found in the web site of the *C. merolae* Genome Project, an EST, esisotig05282, from *P. umbilicalis* and three ESTs, esContig9131, esContig7148 and esContig4510, from *P. purpurea* were obtained and designated PuNCED and PpNCED1 to PpNCED3, respectively. Amino acid sequences and CD-search results of red algal ZEP homologues using the NCBI Conserved Domain Database (CDD; www.ncbi.nlm.nih.gov/cdd) are represented. All Bangiophycean NCED homologues are non-full-length, which prevent good alignment with CLUSTALW.

Fig. S6 Bangiophycean XanDH homologues. Full-length cDNAs encoding XanDH homologues were found from *P. purpurea* and *P. umbilicalis*. For *P. yezoensis* XanDH (Py XanDH), two overlapping but not full-length ESTs, AV439406 and AV438237, were found in the *P. yezoensis* EST database by homology searches with the nucleotide sequence of *A. thaliana* XanDH (AtXanDH; accession no. NM_104113). The amino acid sequence deduced from these ESTs was designated PyXanDH. In addition, two ESTs, esContig7972 of *P. purpurea* and esContig5572 of *P. umbilicalis* were found by homology search at NoriBLAST with the AtXanDH sequence and designated PpXanDH and PuXanDH, respectively. Amino acid sequences and CD-search results of red algal XanDH homologues and AtXanDH, which were obtained using the NCBI Conserved Domain Database (CDD; www.ncbi.nlm.nih.gov/cdd), are represented. In addition, amino acid alignment obtained by CLUSTALW is shown, indicating the presence of the GXXXGXG residues for cofactor binding site and the YXXXK residues for catalytic activity (both are highlighted by red characters), both of which conserved in XanDH (Endo et al. 2014).

Fig. S7 Bangiophycean AAO3 homologues. By homology search at NoriBLAST with AAO3 sequence from *A. thaliana* (AtAAO3; accession no. Q7G9P4), an EST, esFYYDJ9L01CO4XC, from *P. umbilicalis* and five ESTs, esisotig09565, esGAQG33Y01DZ5D6, esGAQG33Y02ILR75, esisotig09716 and esisotig12382, from *P. purpurea* were obtained and designated PuAAO3 and PpAAO3-1 to PpAAO3-5, respectively. Amino acid sequences and CD-search results of red algal AAO3 homologues and AtAAO3, which were obtained using the NCBI Conserved Domain Database (CDD; www.ncbi.nlm.nih.gov/cdd), are represented. All Bangiophycean AAO3 homologues are non-full-length and very short, which prevent good alignment with CLUSTALW.
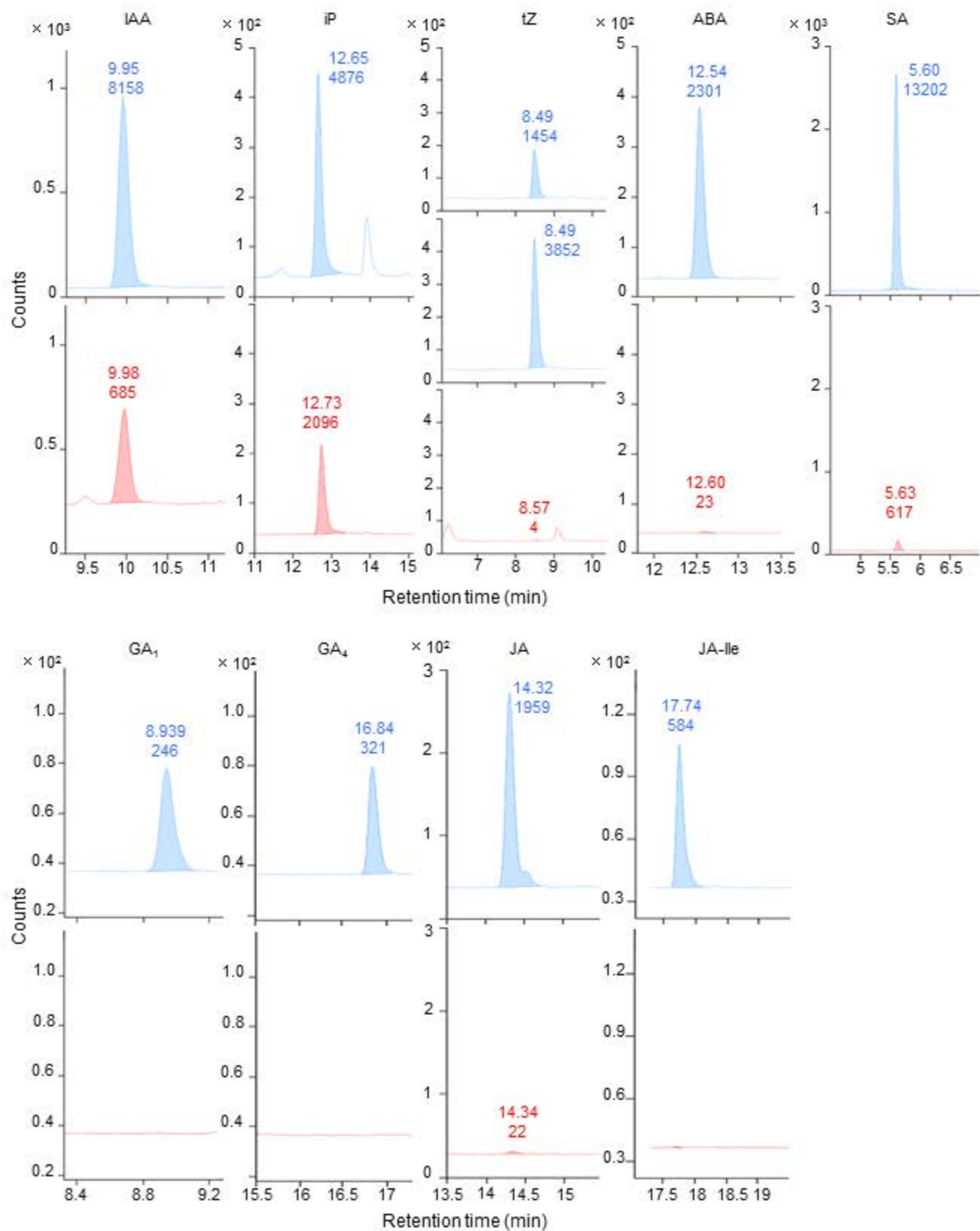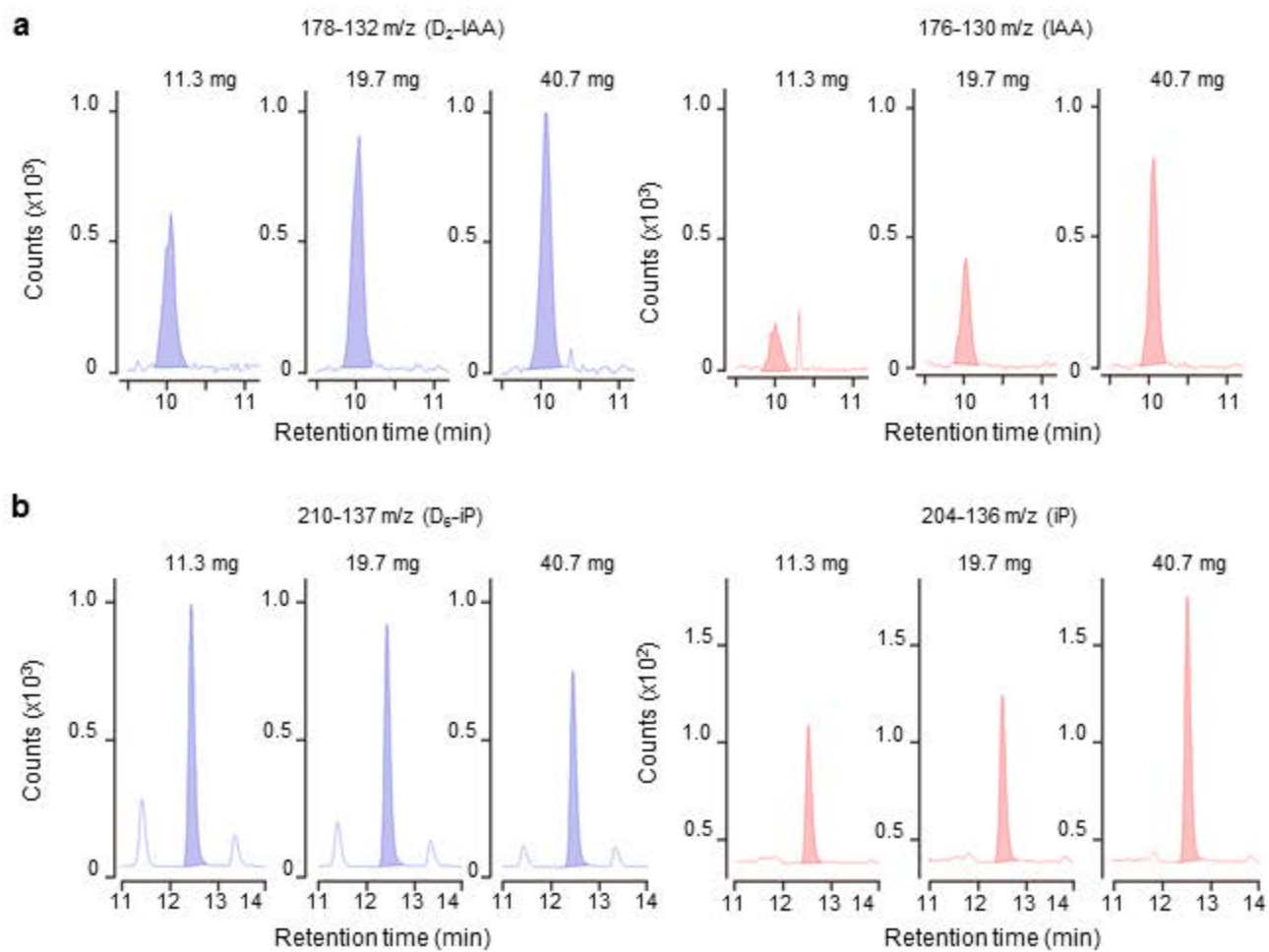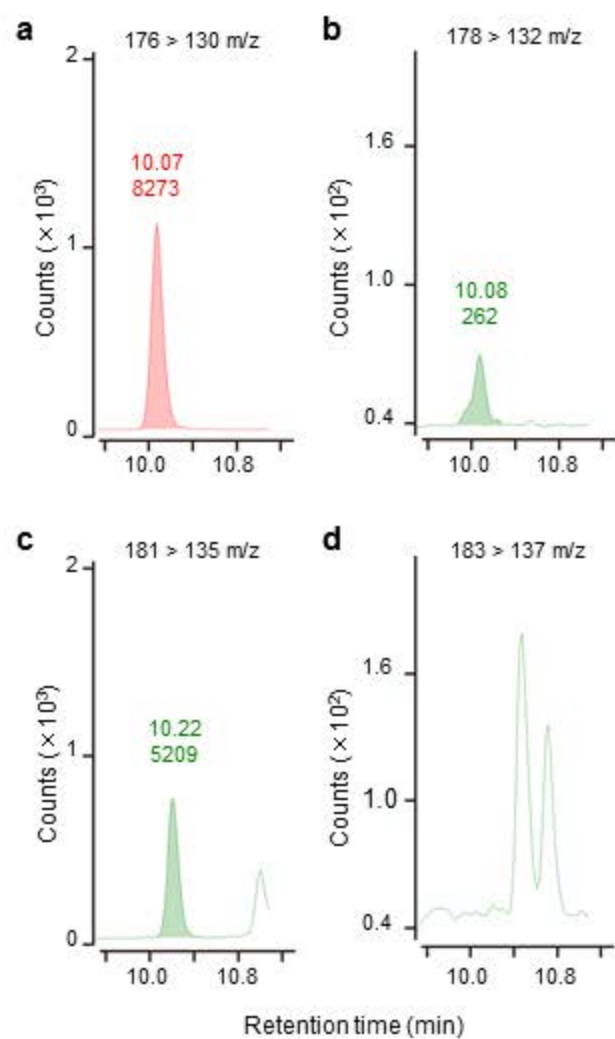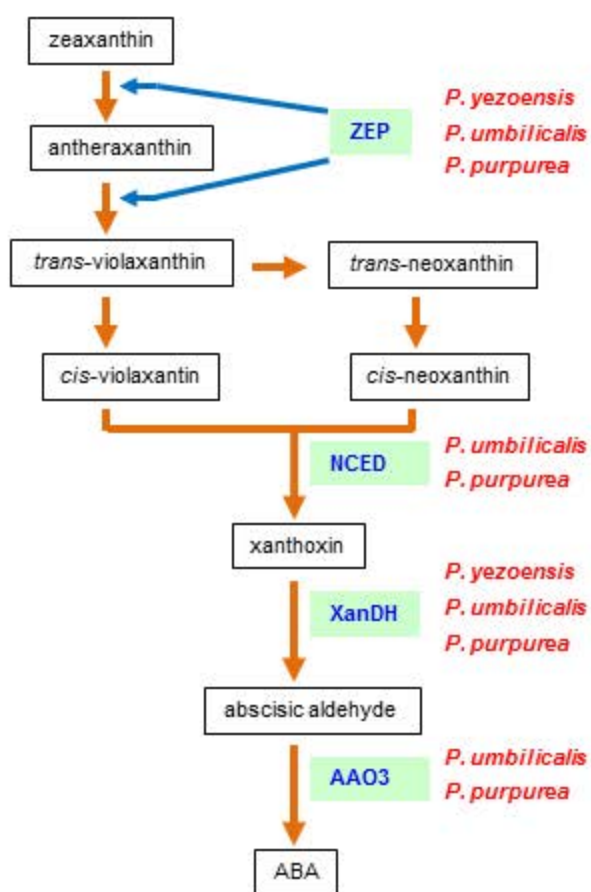
Fig. 1

a

178–132 m/z (D_2-IAA)

11.3 mg   19.7 mg   40.7 mg

176–130 m/z (IAA)

11.3 mg   19.7 mg   40.7 mg

Counts ($\times 10^3$)

Retention time (min)

b

210–137 m/z (D_6-iP)

11.3 mg   19.7 mg   40.7 mg

204–136 m/z (iP)

11.3 mg   19.7 mg   40.7 mg

Counts ($\times 10^3$)

Counts ($\times 10^2$)

Retention time (min)

Fig. 2

a 176 > 130 m/z

Counts ($\times 10^3$)

10.07
8273

10.0   10.8

b 178 > 132 m/z

Counts ($\times 10^2$)

10.08
262

10.0   10.8

c 181 > 135 m/z

Counts ($\times 10^3$)

10.22
5209

10.0   10.8

d 183 > 137 m/z

Counts ($\times 10^2$)

10.0   10.8

Retention time (min)

Fig. 3

Fig. 4

Table S1 List of LC-ESI-MS/MS methods used in the present study

Method No.	Solvent A	Solvent B	Gradient (composition of solvent B)
1	Water containing 0.01% acetic acid	MeCN containing 0.05 % acetic acid	3 to 50 % 20 min
2	Water containing 0.01% acetic acid	MeOH containing 0.2 % acetic acid	3 to 97 % 16 min
3	Water containing 0.1 % formic acid	MeCN containing 0.1 % formic acid	3 to 98 % 10 min

Table S2 Parameters of isotope-labeled internal controls for LC-ESI-MS/MS analysis

|	LC method	Retention time on LC (min)	ESI	MS/MS transitions for quantifications (m/z)	Collision energy (V)	Fragmentor (V)
GA$_1$ D$_2$-GA$_1$ ([^{2}H$_2$]GA1)	1	9.1	-	347/273 349/275	18	160
IAA D$_2$-IAA ([^{2}H$_2$]IAA) D$_5$-IAA ([^{2}H$_5$]IAA) D$_7$-IAA ([^{2}H$_7$]IAA)	1	10	+	176/130 178/132 181/135 183/135,136,137	10	90
ABA D$_6$-ABA ([^{2}H$_6$]ABA)	1	12.6	-	263/153 269/159	5	130
JA D$_2$-JA ([^{2}H$_2$]JA)	1	14.4	-	209/59 211/59	15	135
GA4 D$_2$-GA4 ([^{2}H$_2$]GA4)	1	16.9	-	331.2/257 333.2/259	18	160
JA-IIe ^{13}C$_6$-JA-IIe	1	17.9	-	321.2/130 338.4/136.2	14	140
tZA D$_5$-tZ ([^{2}H$_5$] tZ)	2	8.4	+	220.3/136.3 225.3/136.3,137.3	8	100
iP D$_6$-iP ([^{2}H$_6$]iP)	2	12.5	+	204.4/136.4 210.4/137.4	8	110
SA D$_4$-SA ([^{2}H$_4$]SA)	3	5.6	-	141/97 143/99	12	90

* D = deuterium, **Data in employing ZORBAX Eclipse XDB-C18 column and XDB-C8 Guard column
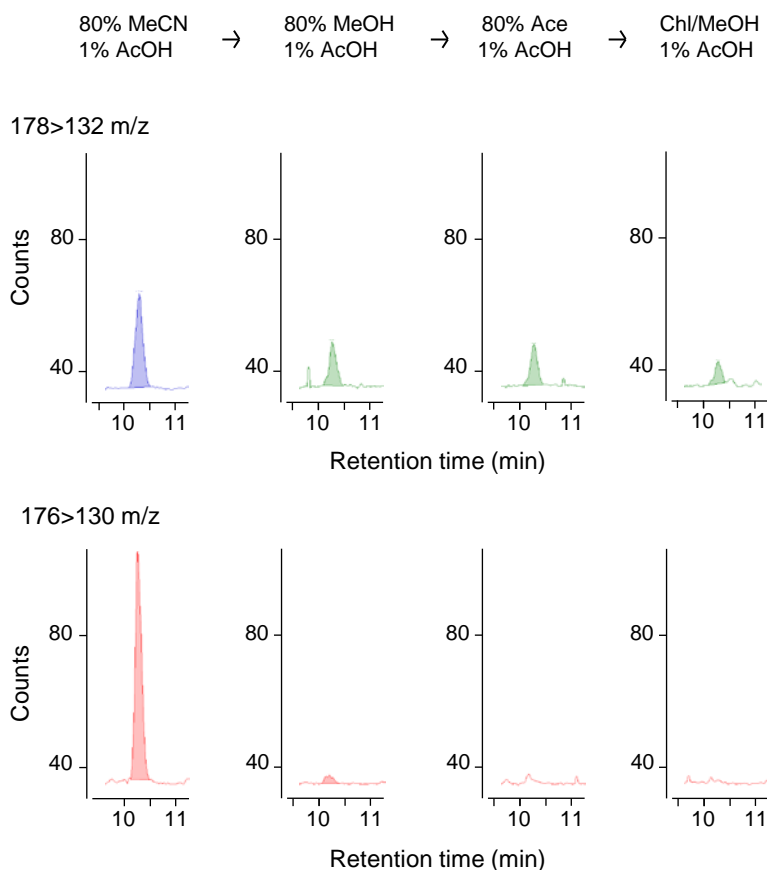
Figure S1. Presence of a cellular substance preventing quantitative analysis of IAA. Sequential stepwise extraction of plant hormones was performed to examine the efficiency of extraction procedure. Ground samples were treated with solvents in the order of 80% acetonitrile containing 1% acetic acid, 80% methanol containing 1% acetic acid, 80% acetone containing 1% acetic acid and chloroform/methanol (1:1) containing 1% acetic acid. Each extract was examined by LC-MS/MS, for which monitoring was done by a MRM chromatogram at178>132 m/z for IAA (upper) and 176>130 m/z for D_2-IAA (lower) and. IAA was completely extracted by the second step, whereas an unidentified cellular substance, which has an MRM transition overlapping that of D_2-IAA, appeared in all extraction steps.

Table S3 Blast search results for cytokinin and ABA biosynthetic genes

Query (Accession no)	Gene name (EST or contig; Clone no)	E-value
AtLOG1 (Q8RUN2)	PpLOG1 (esGAQG33Y01DF64M)	1e-23
	PpLOG2 (esGAQG33Y01DSNGI)	2e-20
	PpLOG3 (esContig5176)	2e-18
AtIPT (NP_177013)	No Blast Hit	
AtCYP735A1 (NP_198661)	No Definite Counterpart	
AtZEP (Q9FGC7)	PuZEP (esisotig05144)	7e-17
	PpZep (esisotig06175)	4e-13
AtVDE (NP_172331)	No Blast Hit	
NECD9 (AEE36100)	PpNCED1 (esContig9131)	6e-19
CmNCED (XP_005538977)	PuNCED (esisotig05282)	5e-11
	PpNCED2 (esContig7148)	3e-12
	PpNCED3 (esContig4510)	2e-11
AtXanDH (NM_104113)	PyXanDH (AV439406, AV438237)	1e-07, 2e-08
	PuXanDH (esContig5572)	3e-13
	PpXanDH (esContig7972)	4e-14
AtAAO3 (Q7G9P4)	PuAAO3 (esFYYDJ9L01CO4XC)	6e-15
	PpAAO3-1 (esisotig09565)	7e-24
	PpAAO3-2 (esGAQG33Y01DZ5D6)	3e-21
	PpAAO3-3 (esGAQG33Y02ILR75)	2e-17
	PpAAO3-4 (esisotig09716)	4e-17
	PpAAO3-5 (esisotig12382)	1e-14
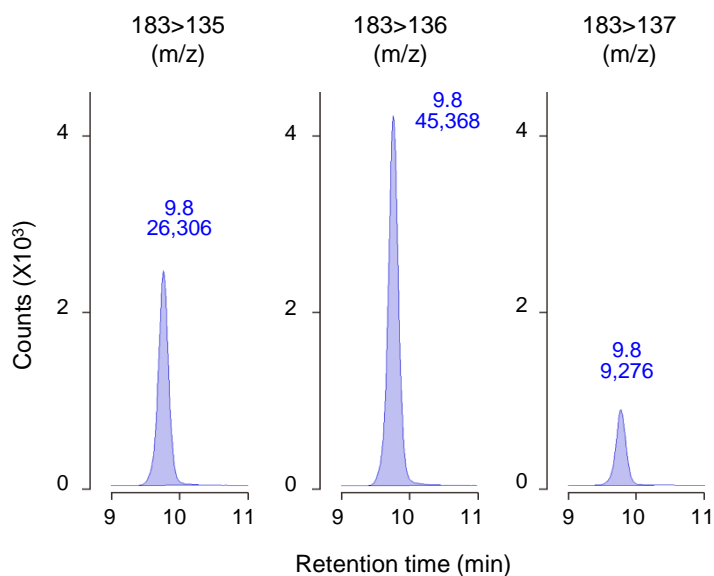
Figure S2. Confirmation of the utility of D_7-IAA as an internal standard. LC-MS/MS performed with MRM at 183>135, 183>136 and 183>137, resulting in detection of three corresponding fragment ions derived from D_7-IAA. As Figure 3 indicates the absence of any cellular substance with MRM transition at 183>137 m/z, D_7-IAA can be used as the internal standard for IAA quantification.
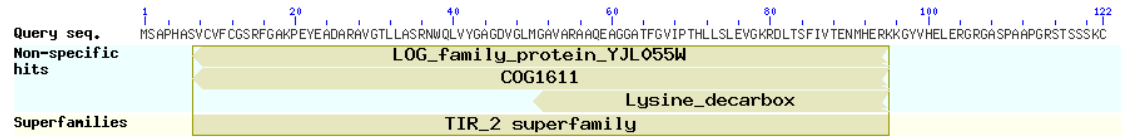
PpLOG1

MSDESGSVAMRELKNICVFCGSQAGNDGSYAAAAAELGKHMVESGIGLVYGGGTVGLMGTIARTVDGAGGSVLGVIPTALSKREI
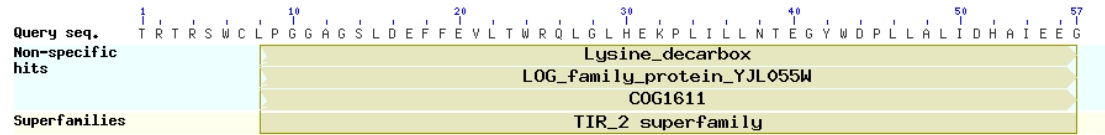SGEGVGTVVEVDDMHTRKAKTRGPKGRRCVHCNARRFGP
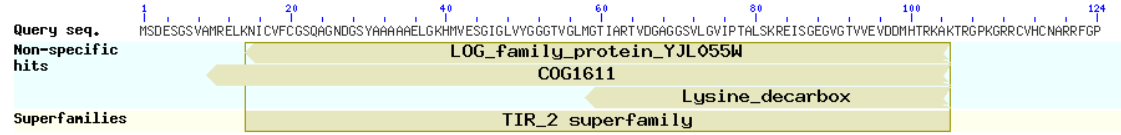
PpLOG2

MSAPHASVCVFCGSRFGAKPEYEADARAVGTLLASRNWQLVYGAGDVGLMGAVARAAQEAGGATFGVIPTHLLSLEVGKRDLTSF
IVTENMHERKKGYVHELERGRGASPAAPGRSTSSSKC

PpLOG3

TRTRSWCLPGGAGSLDEFFEVLTWRQLGLHEKPLILLNTEGYWDPLLALIDHAIEEG

```
AtLOG1   TARHIIVSAPSAKELVKKLEDYVPRHEKVASKKSWEMEQIGLSPTCEISR------------------
AtLOG4   NARQIIISAPTAKELVKKLEEYSPCHESVATKLCWEIERIDYSSED--------------------
OsLOGL2  SARHIIVLAPTPKELIEKLEEYSPQHEKVVSKMKWEMEQMSYPQNYDIPRPKEGKMIIEAQRGSRLWM
AtLOG8   GARNIVVSAPTAKELMEKMEEYTPSHMHVASHESWKVEELGDYPGQENKPQ-----------------
OsLOGL1  DCRQIIVSAPTAHELLRKMEQYTRSHQEVAPRTSWEMSELGYGKTPEES------------------
OsLOGL4  AARNIFVLADNAGELLTKLTEAAAAAAAAAVEGGDGDQVDGEATAAAAGLKRKRS--------------
```

Figure S3. LOG homologues in *P. purpurea*. Three ESTs, esGAQG33Y01DF64M, esGAQG33Y01DSNGI and esContig5176, were found by homology search at NoriBLAST and designated PpLOG1, PpLOG2, and PpLOG3, respectively. These clones are partial and thus full-length information is not yet obtained. In the upper part, amino acid sequences and CD-search results of LOG homologues obtained using the NCBI Conserved Domain Database (CDD; www.ncbi.nlm.nih.gov/cdd) are represented. In the lower part, amino acid alignment obtained by CLUSTALW is shown, by which it is clear that PpLOG1 and PpLOG2 correspond to the N-terminal part of LOG and a short PpLOG3 sequence aligns with the middle part of LOG. Plant LOGs are classified into clade I and clade II (Kuroha et al. 2009). AtLOG1, AtLOG4, and OsLOGL2 belong to clade I, whereas AtLOG8, OsLOGL1, and OsLOGL4 are members of clade II. Since EST information of *P. purpurea* homologues is partial, it is difficult to determine the clade to which they belong. Accession numbers: AtLOG1, Q8RUN2; AtLOG4, Q9LFH3; AtLOG8, Q84MC2; OsLOGL1, Q8LR50; OsLOGL2, B9F166; OsLOGL4, Q851C7.

Reference: Kuroha T, Tokunaga H, Kojima M, Ueda N, Ishida T, Nagawa S, Fukuda H, Sugimoto K, Sakakibara H (2009) Functional analyses of LONELY GUY cytokinin-activating enzymes reveal the importance of the direct activation pathway in *Arabidopsis*. Plant Cell 21: 3152-3169

PuNCED

RATYTGQWVATAQLLAEVRAGFPRYGRLGSMVGAVGAVVMGISSAARAQLGLGLRPAGAGGAPRAGVKRIAAADGSPADAAAGRGT
ANTALVVHAERVLALGEGGLPYALRVACEGVVQTLGSTTFGRGAAGRGAVWTAHPREDAATGELIGVNYHMGAAPYAEVVTVSPA
GAVTSVVPIRSMPRSAMIHDTALTERYVVVLDGPLRLDPARMVRAGTLPFAYHPEQPLRIGLHRRGDAGDGRMAWFSAPNGFIFH
TVAAWEAPDGETVSLVACTYDRVELSLAAGASSPGVLTRYDLHLASGG
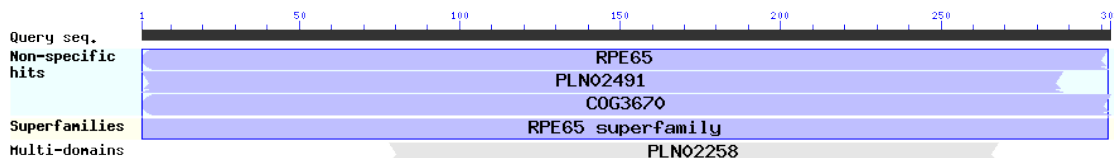
Name	Accession	Description	Interval	E-value
RPE65	pfam03055	Retinal pigment epithelial membrane protein; This family represents a retinal pigment ...	1-302	1.54e-59
PLN02491	PLN02491	carotenoid 9,10(9',10')-cleavage dioxygenase	1-288	3.42e-39
COG3670	COG3670	Lignostilbene-alpha,beta-dioxygenase and related enzymes [Secondary metabolites biosynthesis, ...	1-303	9.88e-39
PLN02258	PLN02258	9-cis-epoxycarotenoid dioxygenase NCED	78-268	6.86e-31

PpNCED1

RPRGSXGGGRQGRVRMRRARRCGGGRGTANTALVVHAKRVLAFGRGASPTPSAWRVRGLSRRLAARPLGGAPPAAGPCGRRYPRE
DASTGELIAVNYYIAAAPHAEVVTVSAGGAVTTVVPRSMPRPAMIHDAAITERFVVVLDGPLRFDPAHMVRAGTLPFAYHPEQPL
RIGLLRRGDDTDGRMAWFSAPNGFIFHTVAAWEAPDRETVTLVACTFDRVDLSFDAGPTAGGVLTRYDLHLPSGGVSTRRLYTTG
GGVDFPRTAATAFWGVGRPPPVRVPLPLCARGGRRRGRPRKVRPLCRGGGGGCGASAGHLWGEATFVPRGSGLGGAAPAAGAAED
DGYLMTYTWTEAAPGVKGAPRRSPCGMRARWRRLRRSAWAARTCRMGSTGCGCRRAGRWGRGRAWRCPLPPTLRRCSACRWGGGG
ARRRRARACRVYIGVTTARPPHAQNSPF
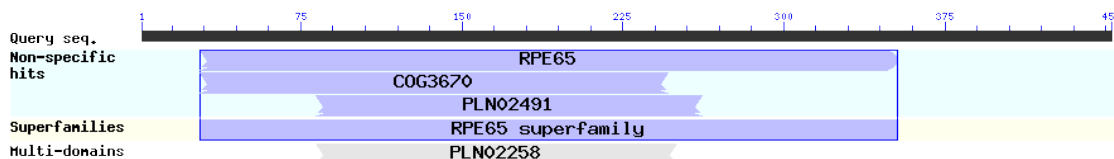
Name	Accession	Description	Interval	E-value
RPE65	pfam03055	Retinal pigment epithelial membrane protein; This family represents a retinal pigment ...	28-353	7.44e-40
COG3670	COG3670	Lignostilbene-alpha,beta-dioxygenase and related enzymes [Secondary metabolites biosynthesis, ...	28-246	1.69e-28
PLN02491	PLN02491	carotenoid 9,10(9',10')-cleavage dioxygenase	82-262	9.33e-26
PLN02258	PLN02258	9-cis-epoxycarotenoid dioxygenase NCED	82-250	5.95e-23

PpNCED2

AALLAGILLLLFDAVCQWLREAVLGRKAPKSEFLKGNFGPVFDELYAEDLPVVGAIPDGLEGQYVRNGPTPRWKPRGQLHWFDGD
GMLHAVLFKEGRATYSNRYVRTQRFLEEEAAEEALHLKIGDWSNPLGKLRSFALISLQKAGFSKLKGRSTANTALAYHGRRFLAL
CESDPPHQVLAPSLDTVGGQFTFGGALATPFTAHPKVDACTGEMFGFGYGFEKPPYLVYFVVG
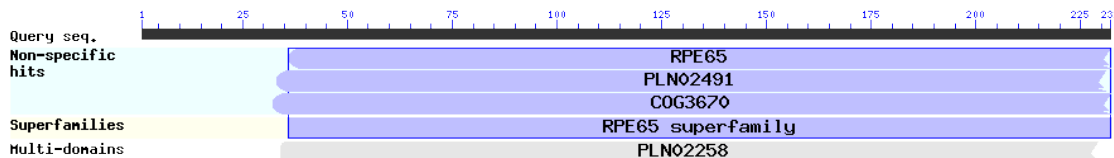
Name	Accession	Description	Interval	E-value
RPE65	pfam03055	Retinal pigment epithelial membrane protein; This family represents a retinal pigment ...	36-232	7.77e-62
PLN02491	PLN02491	carotenoid 9,10(9',10')-cleavage dioxygenase	33-231	5.79e-48
COG3670	COG3670	Lignostilbene-alpha,beta-dioxygenase and related enzymes [Secondary metabolites biosynthesis, ...	32-232	2.00e-46
PLN02258	PLN02258	9-cis-epoxycarotenoid dioxygenase NCED	34-229	5.84e-50

PpNCED3

LTMWRFHRSTGAVERRTLSDTSVEFPVVHPRLLGRKSRYCYCACLDYEPAEPLLNGVVKMDMEKGEVGRITFGEKCYAGEAVFVP
RSEEEDDGYLVTFVHNEATDKSELWVMDAQTMAPEPIARVKIPRRVPYGFHGKWLSAADIADQVTD
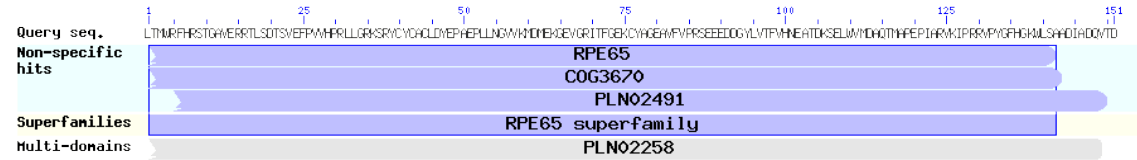
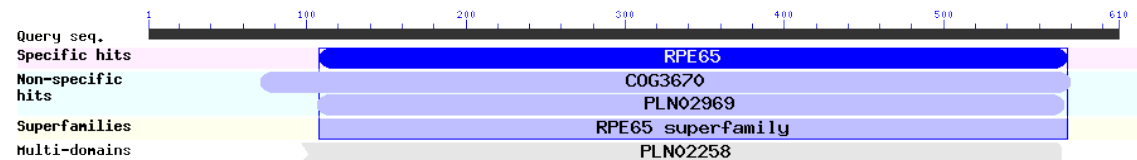| Name	Accession	Description	Interval	E-value
RPE65	pfam03055	Retinal pigment epithelial membrane protein; This family represents a retinal pigment ...	1-142	1.33e-59
COG3670	COG3670	Lignostilbene-alpha,beta-dioxygenase and related enzymes [Secondary metabolites biosynthesis, ...	1-143	8.68e-46
PLN02491	PLN02491	carotenoid 9,10(9',10')-cleavage dioxygenase	5-150	1.78e-35
PLN02258	PLN02258	9-cis-epoxycarotenoid dioxygenase NCED	1-149	3.52e-43

CmNCED

MNLHPGDKTAFVVGNGALRHWKGASVNATATFQCGRLKRRSLRPARSGGATHLSSQESGATNTVREQGQLTSAPGKTQSLSAFDR
RSWELAHGDMNEEDSYVLDLPDLPHDLVGTLYRNGPSKFTIGSRRMLHPWEGDGAVAAFTLRGDGTVWFRNRFVRTEGYLKERRA
GYQLYRGTFATPLPGGIVTNAFRLEQKNLANTNVIYHANQLLALYEGGLPYELRPDTLETKGVFRLGGTLTGPMPMFTAHPHVDP
LRRGRLVGFSSRMHLNGLRIRFLEFASENWKLCSERTVDIDGFGFFHDFMITQRYYILLQAPLRFHPLPFVLGLKCPGECITWEG
DRMPTRLLLIPRDDPGAPVREVLSETCTAFHFVNAYDDDDDDAVVLDACRMDRLFLGETRRHRNGLSRTQRVVEAVDFGTQVPKC
SLWRCRIPTRSGSGTQRATWREVNTTHVDFPVVNPSRLTQPYRYVYMGASALGTEPGPLKNIIKVDADTGQTLATWQPPSVYEFA
GEPVFAPRLIEPDKESAEDDGYILSVVSDGLHRSTYLVILDASDLKLVCKVPLRTFLPMGLHGTWTAQVFQPQPKRRTVQDLFES
KNWNEVDSSLPLFRF

Name	Accession	Description	Interval	E-value
RPE65	pfam03055	Retinal pigment epithelial membrane protein; This family represents a retinal pigment ...	108-578	2.25e-145
COG3670	COG3670	Lignostilbene-alpha,beta-dioxygenase and related enzymes [Secondary metabolites biosynthesis, ...	71-580	3.99e-104
PLN02969	PLN02969	9-cis-epoxycarotenoid dioxygenase	106-576	5.66e-42
PLN02258	PLN02258	9-cis-epoxycarotenoid dioxygenase NCED	97-574	2.56e-36

Figure S5. Bangiophycean NCED homologues. By homology searches at NoriBLAST with NCED sequences from *A. thaliana* (NECD9; accession no. AEE36100) and the unicellular red alga *C. merolae* (CmNCED; CMS362C, accession no. XP_005538977) found in the web site of the *C. merolae* Genome Project, an EST, esisotig05282, from *P. umbilicalis* and three ESTs, esContig9131, esContig7148 and esContig4510, from *P. purpurea* were obtained and designated PuNCED and PpNCED1 to PpNCED3, respectively. Amino acid sequences and CD-search results of red algal ZEP homologues using the NCBI Conserved Domain Database (CDD; www.ncbi.nlm.nih.gov/cdd) are

represented. All Bangiophycean NCED homologues are non-full-length, which prevent good alignment with CLUSTALW.

PyZEP

MAAEQASVAPDAPVSRTVLIAGCGIVGLSTAIALRALPSPPTIIIHERRCKEEAMSGPGGIMIQPNGLAALSALADGSGDTVVAA
ISAVARPLGSGGFQSATGEDLYIAEPARVTFGARTSDLGVSVSRTALMGALSTAAGVGAPGGVEVVWQSAVRHFEVVQGEEGVLK
VDVQLETAGVTQMVRGVDVLVGADGLWSGVRSAVDEVTGRVPTPRTYGGLMWWRGSVSYSAVEARCADIGRLSWAQAWLPRGASL
GYIRLGGDDLAWFASAPRPADAPVEGGSPLAEFLAEFGPDRGTTPPVYAAVVAELASRGEAADCIYRGPIYSRGVPSGPGWGVGP
VTLVGDAAHAIFPSMGQGACVGIEDAVELAACLAAAWRSQPLVGAAAAASRGTVPAALRAFEAARAPRVTRMGVESARVYHLSAL
TAAPGIWLRDTAYSMLPQWVVDRQFSWLFSYKATVVA
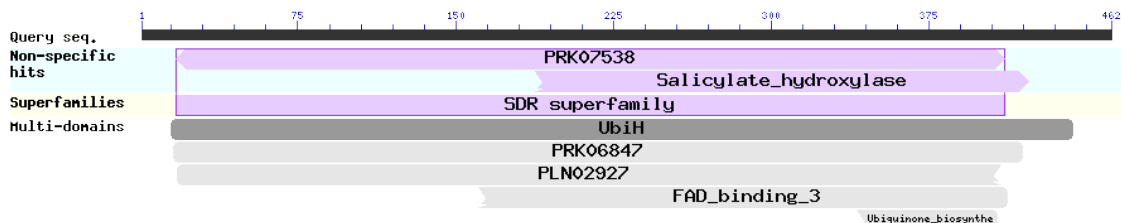
Name	Accession	Description	Interval	E-value
PRK07538	PRK07538	hypothetical protein; Provisional	17-411	4.81e-18
Salicylate_hydroxylase	TIGR03219	salicylate 1-monooxygenase; Members of this protein family are salicylate 1-monooxygenase, ...	188-422	1.71e-11
UbiH	COG0654	2-polyprenyl-6-methoxyphenol hydroxylase and related FAD-dependent oxidoreductases [Coenzyme ...	15-443	1.97e-35
PRK06847	PRK06847	hypothetical protein; Provisional	16-419	2.65e-25
PLN02927	PLN02927	antheraxanthin epoxidase/zeaxanthin epoxidase	18-409	5.15e-20
FAD_binding_3	pfam01494	FAD binding domain; This domain is involved in FAD binding in a number of enzymes.	161-412	9.75e-09
Ubiquinone_biosynthesis_monooxygenase_COQ6	TIGR01988	Ubiquinone biosynthesis hydroxylase, UbiH/UbiF/VisC/COQ6 family; This model represents a ...	341-407	2.10e-05

PpZEP

LVSPLYRLSFCPVFVRLSASTPGVVPAPAAEHGFFPSLSDCRYCWGWYRWAHHRHIAASAATPPTVIIHERRPEETALSGPGGIL
IQLNGLAALSALADGSGEALIAALGAVSCPIGPGGFQSAAGKDLYIADPVSVTLGRRTNDVGVSVSRTALMGVLAEAAKIGQPGG
VEVVWESAVTGYATTDGAVDQVDVQVQTAGQAATIRGVDALIGADGIWSGVRSAVDAVVGRPSTPPRYGGLMWWRGSVSYSAVAA
RCADVGRLSWGQAWLPRGTSMGYFRLGGDGMAWFASAPRPADEPVDGSPLAELLAEFGPSRGTSPPVYAAIVDAVAAAGSDTINR
APIYSRGVPAAAAGVGPVWGTGPVTLAGDAAHAIFPSLGQGACIGMEDAAEVAAALATAWRDGRPGSVPAALRAYEAARAPRVTR
VGAESGRVYDLSALTGGVSVWLRDTVYSVLPQWLVDKQFSWLYDYKPTPVPVA
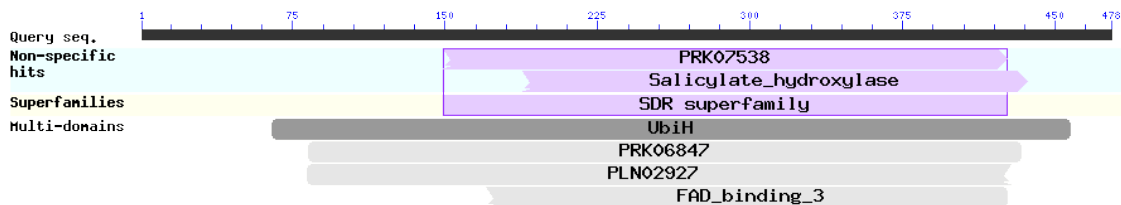
Name	Accession	Description	Interval	E-value
PRK07538	PRK07538	hypothetical protein; Provisional	149-426	5.49e-14
Salicylate_hydroxylase	TIGR03219	salicylate 1-monooxygenase; Members of this protein family are salicylate 1-monooxygenase, ...	188-436	1.13e-09
UbiH	COG0654	2-polyprenyl-6-methoxyphenol hydroxylase and related FAD-dependent oxidoreductases [Coenzyme ...	65-457	8.02e-25
PRK06847	PRK06847	hypothetical protein; Provisional	83-433	1.98e-20
PLN02927	PLN02927	antheraxanthin epoxidase/zeaxanthin epoxidase	82-428	3.73e-13
FAD_binding_3	pfam01494	FAD binding domain; This domain is involved in FAD binding in a	170-426	1.74e-05

PuZEP

```
ILSIGLAPLSPVTSSFGRQRRRSSSPLLPPCLFCHVLVSLSASIPGGVPAPVPEHGVFFPPPRTVVIAGAGIVGLTTAISLRALP
TPPTVIIHERRPEEGALSGPGGIMIQPNGLAALSALADGSGEAVVAALNAVSCPLGRGGFQSATGKDLYIAEPAGVTLGERTDDL
GVCVARTALMGVLAEAAKIGQPGGVEVVWESAVTSYTTTDGAVDKVDVHVKTSGQAATIRGVDVLIGADGLWSGVRSAVDAVTGR
PSTPRHYGGLMWWRGSVSFSAVAARCADIGRLAWGQAWLPRGGSMGYFRLGGDDMAWYASAPRPADAPVDGSPLAELLAEFGPTR
GTTPPVYAAIVDAVAAAGSDTINRAPVYSRGVPVAAAGVGPVWGTGPVTLVGDAAHAIFPSMGQGACVGMEDAAELAAALAAAWR
DGQPGGVPAALRAYEAARAPRVTRVGVESARVYDLSALTGGVSIWLRDTVYSVLPQWIVDRQFSWLYDYKPTPVLVA
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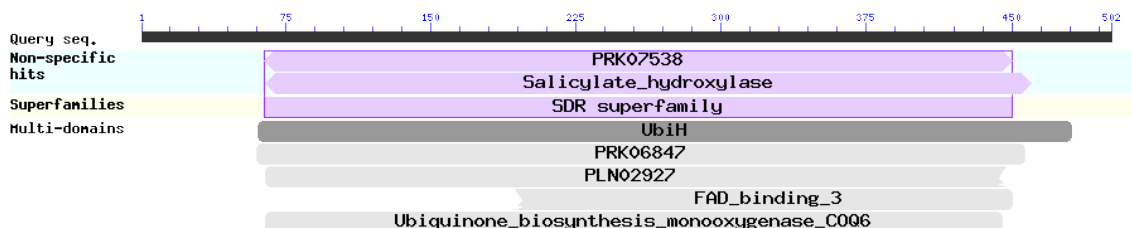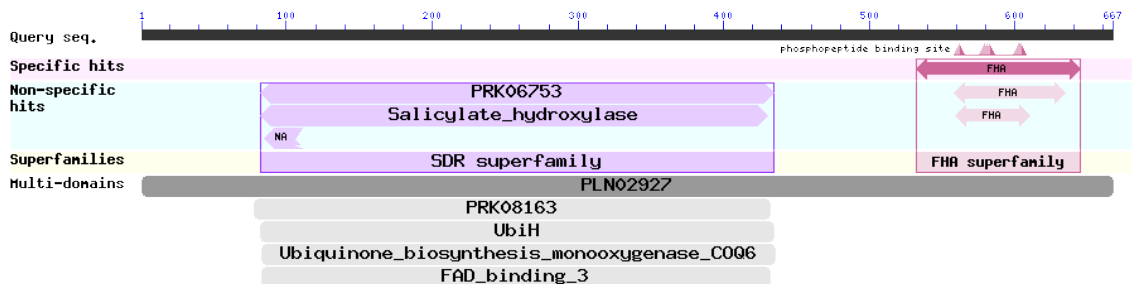

Name	Accession	Description	Interval	E-value
PRK07538	PRK07538	hypothetical protein; Provisional	64-450	1.22e-21
Salicylate_hydroxylase	TIGR03219	salicylate 1-monooxygenase; Members of this protein family are salicylate 1-monooxygenase, ...	65-460	5.02e-09
UbiH	COG0654	2-polyprenyl-6-methoxyphenol hydroxylase and related FAD-dependent oxidoreductases [Coenzyme ...	61-481	2.04e-33
PRK06847	PRK06847	hypothetical protein; Provisional	60-457	1.71e-23
PLN02927	PLN02927	antheraxanthin epoxidase/zeaxanthin epoxidase	65-447	1.24e-17
FAD_binding_3	pfam01494	FAD binding domain; This domain is involved in FAD binding in a number of enzymes.	194-450	1.50e-07
Ubiquinone_biosynthesis_monooxygenase_COQ6	TIGR01988	Ubiquinone biosynthesis hydroxylase, UbiH/UbiF/VisC/COQ6 family; This model represents a ...	65	

AtZEP

```
MGSTPFCYSINPSPSKLDFTRTHVFSPVSKQFYLDLSSFSGKPGGVSGFRSRRALLGVKAATALVEKEEKREAVTEKKKKSRVLV
AGGGIGGLVFALAAKKKGFDVLVFEKDLSAIRGEGKYRGPIQIQSNALAALEAIDIEVAEQVMEAGCITGDRINGLVDGISGTWY
VKFDTFTPAASRGLPVTRVISRMTLQQILARAVGEDVIRNESNVVDFEDSGDKVTVVLENGQRYEGDLLVGADGIWSKVRNNLFG
RSEATYSGYTCYTGIADFIPADIESVGYRVFLGHKQYFVSSDVGGGKMQWYAFHEEPAGGADAPNGMKKRLFEIFDGWCDNVLDL
LHATEEEAILRRDIYDRSPGFTWGKGRVTLLGDSIHAMQPNMGQGGCMAIEDSFQLALELDEAWKQSVETTTPVDVVSSLKRYEE
SRRLRVAIIHAMARMAAIMASTYKAYLGVGLGPLSFLTKFRVPHPGRVGGRFFVDIAMPSMLDWVLGGNSEKLQGRPPSCRLTDK
ADDRLREWFEDDDALERTIKGEWYLIPHGDDCCVSETLCLTKDEDQPCIVGSEPDQDFPGMRIVIPSSQVSKMHARVIYKDGAFF
LMDLRSEHGTYVTDNEGRRYRATPNFPARFRSSDIIEFGSDKKAAFRVKVIRKTPKSTRKNESNNDKLLQTA
```


Name	Accession	Description	Interval	E-value
FHA	cd00060	Forkhead associated domain (FHA); found in eukaryotic and prokaryotic proteins. Putative ...	532-645	2.65e-17

PRK06753	PRK06753	hypothetical protein; Provisional	82-434	2.00e-49
Salicylate_hydroxylase	TIGR03219	salicylate 1-monooxygenase; Members of this protein family are salicylate 1-monooxygenase, ...	82-430	2.69e-16
FHA	pfam00498	FHA domain; The FHA (Forkhead-associated) domain is a phosphopeptide binding motif.	558-634	3.42e-13
FHA	smart00240	Forkhead associated domain; Found in eukaryotic and prokaryotic proteins. Putative nuclear ...	559-610	4.38e-07
NAD_binding_8	pfam13450	NAD(P)-binding Rossmann-like domain;	85-111	1.34e-05
PLN02927	PLN02927	antheraxanthin epoxidase/zeaxanthin epoxidase	1-667	0e+00
PRK08163	PRK08163	salicylate hydroxylase; Provisional	78-432	5.74e-48
UbiH	COG0654	2-polyprenyl-6-methoxyphenol hydroxylase and related FAD-dependent oxidoreductases [Coenzyme ...	82-434	2.28e-46
Ubiquinone_biosynthesis_monooxygenase_COQ6	TIGR01988	Ubiquinone biosynthesis hydroxylase, UbiH/UbiF/VisC/COQ6 family; This model represents a ...	83-435	1.39e-14
FAD_binding_3	pfam01494	FAD binding domain; This domain is involved in FAD binding in a number of enzymes.	83-432	1.82e-12

```
PuZEP    ----------------------ILSIGLAPLSPVTSSFGRQRRRSSSPLLPPCLFCHVL
PpZEP    --------------------------------------------LVSPLYRLSFCPVF
PyZEP    ----------------------------------------------------------
OsZEP    ---MALLSATAPAKTRFS-----LFSHEEAQHPHPHALSACCGGGASGKRQRARARVAAA
ZmZEP    ---MALLSATSPAKAHLPALLVYHDEPQQHQHALPPAHPQCFGGGGGKARQRARGRCAAA
AtZEP    MGSTPFCYSINPSPSKLDFTRTHVFSPVSKQFYLDLSSFSGKPGGVSGFRSRR-----AL


PuZEP    VSLSASIPGGVPAPVPEHGVFFPPPRTVVIAGAGIVGLTTAISLRALPTPPTVIIHERRP
PpZEP    VRLSASTPGVVPAPAAEHGFFPSLSDCRYCWGWYRWAHHRHIAASAA-TPPTVIIHERRP
PyZEP    ----------MAAEQASVAPDAPVSRTVLIAGCGIVGLSTAIALRALPSPPTIIIHERRC
OsZEP    MRPADAAASVAQAASPGGGGEGTRRPRVLVAGGGIGGLVALAARRKGYEVTVFERDMSA
ZmZEP    MRPLDAAVAPTPTPAAG--EAPRKRPRVLVAGGGIGGLVALAARRKGYDVTVFERDLSA
AtZEP    LGVKAATALVEKEEKREAVTEKKKKSRVLVAGGGIGGLVFALAAKKKGFDVLVFEKDLSA
                              *     .    ::          :: ::


PuZEP    EEGALSGPGGIMIQPNGLAALSALADGSGEAVVAALNAVSCPLGRGGFQSATGKDLYIAE
PpZEP    EETALSGPGGILIQLNGLAALSALADGSGEALIAALGAVSCPIGPGGFQSAAGKDLYIAD
PyZEP    KEEAMSGPGGIMIQPNGLAALSALADGSGDTVVAAISAVARPLGSGGFQSATGEDLYIAE
OsZEP    VRGEGQYRGPIQIQSNALAALAAIDMSVAEEVMREGCVTGDRIN--GLVDGISGSWYIKF
ZmZEP    VRGEGQYRGPIQIQSNALAALEAIDMSVAEEVMRVGCVTGDRIN--GLVDGMSGSWYIKF
AtZEP    IRGEGKYRGPIQIQSNALAALEAIDIEVAEQVMEAGCITGDRIN--GLVDGISGTWYVKF
           .   .  * * ** *.**** *:     .: ::     .. :. *:.. .    *:


PuZEP    PAGVTLGERTDDLGVCVARTALMGVLAEAAKIGQPGGVEVVWESAVTSYTTTDG--AVDK
PpZEP    PVSVTLGRRTNDVGVSVSRTALMGVLAEAAKIGQPGGVEVVWESAVTGYATTDG--AVDQ
PyZEP    PARVTFGARTSDLGVSVSRTALMGALSTAAGVGAPGGVEVVWQSAVRHFEVVQGEEGVLK
OsZEP    DTFTPAAERGLPVTRVISRMTLQQILARAVGDDAILNDSHVVDFIDD-------------
ZmZEP    DTFTPAAERGLPVTRVISRMTLQQILARAVGNDAILNGSHVVDFIDD-------------
AtZEP    DTFTPAASRGLPVTRVISRMTLQQILARAVGEDVIRNESNVVDFEDS-------------
           .  . . *   :     ::* :*   *: *.  .   . . * :


PuZEP    VDVHVKTSGQAATIRGVDVLIGADGLWSGVRSAVDAVTGRPSTPRHYGGLMWWRGSVSFS
PpZEP    VDVQVQTAGQAATIRGVDALIGADGIWSGVRSAVDAVVGRPSTPPRYGGLMWWRGSVSYS
PyZEP    VDVQLETAGVTQMVRGVDVLVGADGLWSGVRSAVDEVTGRVPTPRTYGGLMWWRGSVSYS
```

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OsZEP  -GNKVTAILEDGRKFEGDLLVGADGIWSKVR-----KVLFGQSEATYSEYTCYTGIADFV
ZmZEP  -GSKVTAILEDGRKFEGDLLVGADGIWSKVR-----KTLFGHSDATYSGYTCYTGIADFV
AtZEP  -GDKVTVVLENGQRYEGDLLVGADGIWSKVR-----NNLFGRSEATYSGYTCYTGIADFI
         . ::.         * *:****:** **        :    *.    : * ..:


PuZEP  AVAARCADIGRLAWGQAWLPRGGSMGYFRLGGDDMAWYASAPRPADAPVDG-SPLAELLA
PpZEP  AVAARCADVGRLSWGQAWLPRGTSMGYFRLGGDGMAWFASAPRPADEPVDG-SPLAELLA
PyZEP  AVEARCADIGRLSWAQAWLPRGASLGYIRLGGDDLAWFASAPRPADAPVEGGSPLAEFLA
OsZEP  PPDIDTVGYRVFLGHKQYFVSS------DVGAGKMQWYAFHKEPAGGTDPENGKNKRLLE
ZmZEP  PPDIDTVGYRVFLGHKQYFVSS------DVGAGKMQWYAFHNEEAGGTDPENGKKKKLLE
AtZEP  PADIESVGYRVFLGHKQYFVSS------DVGGGKMQWYAFHEEPAGGADAPNGMKKRLFE
         .      .. :   : ::  .       :*.. : *:*  . *.  .    .  .::


PuZEP  EFGPTRGTTPPVYAAIVDAVAAAG--SDTINRAPVYSRGVPVAAAGVGPVWGTGPVTLVG
PpZEP  EFGPSRGTSPPVYAAIVDAVAAAG--SDTINRAPIYSRGVPAAAAGVGPVWGTGPVTLAG
PyZEP  EFGPDRGTTPPVYAAVVAELASRGEAADCIYRGPIYSRGVPSGPG-----WGVGPVTLVG
OsZEP  IFNGWCDNVVDLINATDEEAILRR---DIYDRPPTFN------------WGKGRVTLLG
ZmZEP  IFDGWCDNVIDLINATDEEAVLRR---DIYDRPPTMN------------WGKGRVTLLG
AtZEP  IFDGWCDNVLDLLHATEEEAILRR---DIYDRSPGFT------------WGKGRVTLLG
        *.  ..   :  *     *   * .          ** * *** *


PuZEP  DAAHAIFPSMGQGACVGMEDAAELAAALAAAWRD---------GQPGGVPAALRAYEAAR
PpZEP  DAAHAIFPSLGQGACIGMEDAAEVAAALATAWRD---------GRPGSVPAALRAYEAAR
PyZEP  DAAHAIFPSMGQGACVGIEDAVELAACLAAAWRSQPLVGAAAAASRGTVPAALRAFEAAR
OsZEP  DSVHAMQPNLGQGGCMAIEDGYQLAVELEKSWQES-----AKSGTPMDIVSSLRRYEKER
ZmZEP  DSVHAMQPNLGQGGCMAIEDGYQLAVELENAWQES-----VKTETPIDIVSSLRRYEKER
AtZEP  DSIHAMQPNMGQGGCMAIEDSFQLALELDEAWKQS-----VETTTPVDVVSSLKRYEESR
        *: **: *.:***.*:.:**. ::* * :*:.        : ::*: :* *


PuZEP  APRVTRVG----VESARVYDLSALTGG--------------------------------
PpZEP  APRVTRVG----AESGRVYDLSALTGG--------------------------------
PyZEP  APRVTRMG----VESARVYHLSALTAA--------------------------------
OsZEP  ILRVSVIHGLARMAAIMATTYRPYLGVGLGPLSFLTKLRIPHPGRVGGRFFIKYGMPLML
ZmZEP  RLRVAIIHGLARMAAIMATTYRPYLGVGLGPLSFLTKLRIPHPGRVGGRFFIKYGMPAML
AtZEP  RLRVAIIHAMARMAAIMASTYKAYLGVGLGPLSFLTKFRVPHPGRVGGRFFVDIAMPSML
         **: :          :   .    .  .


PuZEP  ---------------VSIWLRDTVYSVLPQWIVDR------------------------
PpZEP  ---------------VSVWLRDTVYSVLPQWLVDK------------------------
PyZEP  ---------------PGIWLRDTAYSMLPQWVVDR------------------------
OsZEP  SWVLGGNSTKLEGRPLSCRLSDKANDQLRRWFEDDDALEQAMGGEWYLLPTSSGDS---Q
ZmZEP  SWVLGGNSSKLEGRLLSCRLSDKANDQLYQWFEDDDALEEAMGGEWYLIATSEGNCNSLQ
AtZEP  DWVLGGNSEKLQGRPPSCRLTDKADDRLREWFEDDDALERTIKGEWYLIPHG-DDCCVSE
          .  * *.. . * .*. *


PuZEP  -----------------------------------------------------QFSW
PpZEP  -----------------------------------------------------QFSW
PyZEP  -----------------------------------------------------QFSW
OsZEP  PIRLIRDEKKSLSIGSRSDPSNSTASLALPLPQISENHATITCKNKAFYVTDNGSEHGTW
ZmZEP  PIHLIRDEQRSLFVGSRSDPNDSASSLSLSSPQISERHATITCKNKAFYLTDLGSEHGTW
AtZEP  TLCLTKDEDQPCIVGSEPDQDFPGMRIVIPSSQVSKMHARVIYKDGAFFLMDLRSEHGTY


PuZEP  LYDYKPTPVLVA--------------------------------------------
PpZEP  LYDYKPTPVPVA--------------------------------------------
PyZEP  LFSYKATVVA----------------------------------------------
OsZEP  ITDNEGRRYRRTSELPCPFPSLGCH-------------------------------
```

```
ZmZEP        ITDNEGRRYRVPPNFPVRFHPSDVIEFGSDKKAMFRVKVLNTLPYESARSGNRQQQQVLQAA
AtZEP        VTDNEGRRYRATPNFPARFRSSDIIEFGSDKKAAFRVKVIRKTPKSTRKNESNNDKLLQTA
```

Figure S4. Bangiophycean ZEP homologues. The cDNAs encoding ZEP homologues were identified in *P. yezoensis*, *P. purpurea* and *P. umbilicalis*. For *P. yezoensis* ZEP (PyZEP), the full-length information was obtained as contig19911_g4901 from the *P. yezoensis* genome information by searching with conserved amino acid sequence of ZEP. Two ESTs, esisotig06175 of *P. purpurea* and esisotig05144 of *P. umbilicalis* were found by homology searches at NoriBLAST with the ZEP sequence from *A. thaliana* (AtZEP) and designated PpZEP and PuZEP, respectively. Amino acid sequences and CD-search results of red algal ZEP homologues and AtZEP, which were obtained using the NCBI Conserved Domain Database (CDD; www.ncbi.nlm.nih.gov/cdd), are represented. In addition, amino acid alignment obtained by CLUSTALW is shown. According to the above information, it is clear that Bangiophycean ZEP homologues lack the Forkhead-associated (FHA) domain found at the C-terminal region of AtZEP, suggesting differences in regulatory mode in ZEP function between red seaweeds and terrestrial plants. Accession numbers: AtZEP, Q9FGC7; OsZEP from rice, Q0JCU7; ZmZEP from corn, ACG42893.

PyXanDH

AAKRDVCYPICFDPSNSEAVTAAVARIDADAWPVYVLINNSGILSNTLVEETSIDEWRNVFAQNVDSAFLLSRALLPAMRKRKWG
RIVNTCSLAGKTGGVTTGVAYSTTKGALQTLTFALARESAKDGITVNGIAPAYIRTPTVETYPEDVVDMLLRYIPVSRFCEPEEF
AHVVRFLISPMSGFITGEIIDQNGGLHMS
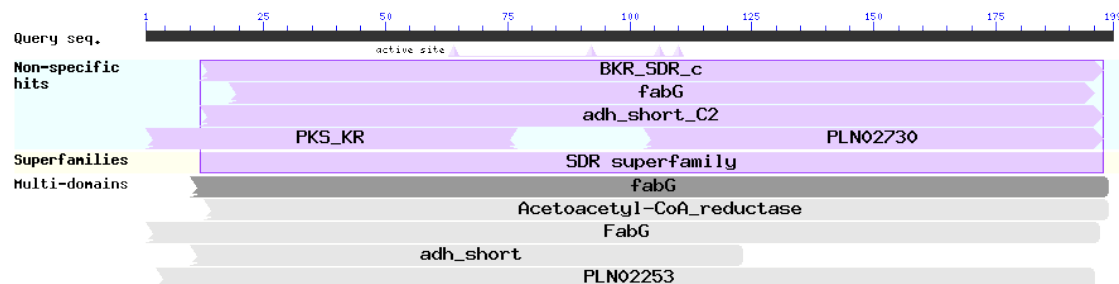
Name	Accession	Description	Interval	E-value
BKR_SDR_c	cd05333	beta-Keto acyl carrier protein reductase (BKR), involved in Type II FAS, classical (c) SDRs; ...	12-197	2.49e-58
fabG	PRK06550	3-ketoacyl-(acyl-carrier-protein) reductase; Provisional	18-195	1.59e-35
adh_short_C2	pfam13561	Enoyl-(Acyl carrier protein) reductase;	12-197	2.88e-14
PKS_KR	smart00822	This enzymatic domain is part of bacterial polyketide synthases; It catalyses the first step ...	1-77	3.26e-05
PLN02730	PLN02730	enoyl-[acyl-carrier-protein] reductase	103-197	4.28e-05
fabG	PRK05653	3-ketoacyl-(acyl-carrier-protein) reductase; Validated	10-198	7.15e-62
Acetoacetyl-CoA_reductase	TIGR01829	acetoacetyl-CoA reductase; This model represent acetoacetyl-CoA reductase, a member of the ...	13-198	2.42e-54
FabG	COG1028	Dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases) ...	1-196	5.55e-42
adh_short	pfam00106	short chain dehydrogenase; This family contains a wide variety of dehydrogenases.	10-123	6.16e-21
PLN02253	PLN02253	xanthoxin dehydrogenase	3-195	7.16e-11

PuXanDH

MSVPRLPPLAAAMARSAAAAASSSVPAFAATAGVPLGSVAARATASASTTAAVCRAGAGSLFGTPLRGRAAAVAAAAAGRLPSTP
STSATTTTTIKTTTTTRGVPSMAADGSPKVAIVTGASRGIGKAIALALAAAGCKVVVNYARSADAAASVVADIEAAGGEALAVRG
DVSQAADVAALFQTVVATYGAVDVLVNNAGIITDTLLMRMKQAQWQEVIDLNLTGVFLCTQAAAKLMIKARAGRIVNIASVVGQIG
NPGQVNYAAAKGGVLGLTMATAKEVASRGVTVNAVAPGFIASDMTAELPLDKIKAMIPMGRLGEAGRGGGHGHLPRHRPGRGVRD
GAHV
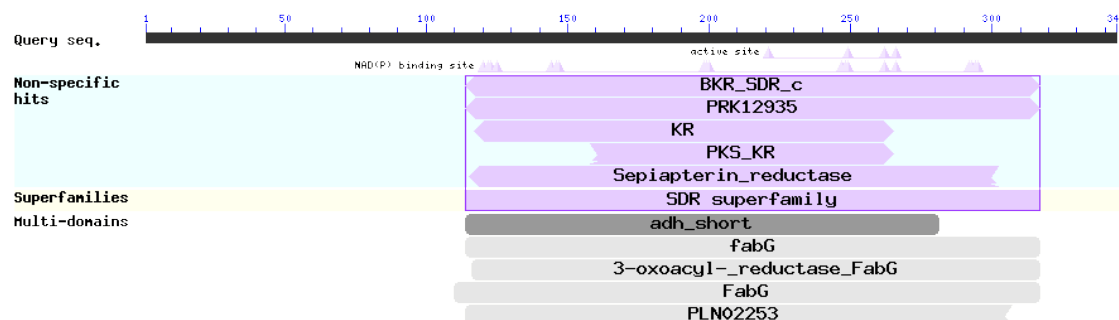
Name	Accession	Description	Interval	E-value
BKR_SDR_c	cd05333	beta-Keto acyl carrier protein reductase (BKR), involved in Type II FAS, classical (c) SDRs; ...	114-317	2.00e-92
PRK12935	PRK12935	acetoacetyl-CoA reductase; Provisional	114-317	3.95e-53
KR	pfam08659	KR domain; This enzymatic domain is part of bacterial polyketide synthases and catalyses the ...	117-265	2.53e-10
PKS_KR	smart00822	This enzymatic domain is part of bacterial polyketide synthases; It catalyses the first step ...	158-265	3.85e-09

|				
Sepiapterin_reductase	TIGR01500	sepiapterin reductase; This model describes sepiapterin reductase, a member of the short chain ...	115-302	1.37e-05
adh_short	pfam00106	short chain dehydrogenase; This family contains a wide variety of dehydrogenases.	114-281	6.31e-31
fabG	PRK05557	3-ketoacyl-(acyl-carrier-protein) reductase; Validated	114-317	1.62e-91
3-oxoacyl-_reductase_FabG	TIGR01830	3-oxoacyl-(acyl-carrier-protein) reductase; This model represents 3-oxoacyl-[ACP] reductase, ...	116-317	1.29e-88
FabG	COG1028	Dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases) ...	110-317	1.08e-49
PLN02253	PLN02253	xanthoxin dehydrogenase	114-307	2.12e-24

PpXanDH

MAPPPPDAAGVNPSAASRMDLTGKRALVTGGHRGIGAAIVLGLAAAGAHVAVIDRGGNASRTSDIPSRVRGYGVEFFAAAADLGD
PEQVMAVVSRVLSEFGNIDILVNNAGITELQPLTTTPVHPDPPLSVLAWDAQFAVNVRAPMLLAQAVAPAMVAARRGKIINVTSQ
AGTAALPLHAAYGASKAALDHLTRGMVAEWGRYNIQANAVAPTVIASDMGDAAWPPGAPKTVTMLDRIPAGRFGQAWEVADLVVF
LAGGGAT
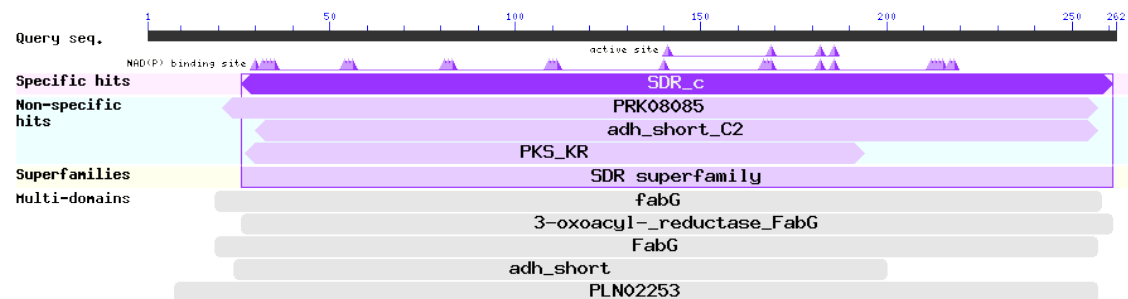
Name	Accession	Description	Interval	E-value
SDR_c	cd05233	classical (c) SDRs; SDRs are a functionally diverse family of oxidoreductases that have a ...	26-261	3.06e-63
PRK08085	PRK08085	gluconate 5-dehydrogenase; Provisional	21-257	1.53e-41
adh_short_C2	pfam13561	Enoyl-(Acyl carrier protein) reductase;	30-257	2.74e-16
PKS_KR	smart00822	This enzymatic domain is part of bacterial polyketide synthases; It catalyses the first step ...	27-194	4.53e-09
fabG	PRK05557	3-ketoacyl-(acyl-carrier-protein) reductase; Validated	19-258	7.54e-62
3-oxoacyl-_reductase_FabG	TIGR01830	3-oxoacyl-(acyl-carrier-protein) reductase; This model represents 3-oxoacyl-[ACP] reductase, ...	26-261	2.55e-55
FabG	COG1028	Dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases) ...	19-257	4.02e-54
adh_short	pfam00106	short chain dehydrogenase; This family contains a wide variety of dehydrogenases.	24-200	9.18e-31
PLN02253	PLN02253	xanthoxin dehydrogenase	8-257	8.28e-20

AtXanDH

MSTNTESSSYSSLPSQRLLGKVALITGGATGIGESIVRLFHKHGAKVCIVDLQDDLGGEVCKSLLRGESKETAFFIHGDVRVEDD
ISNAVDFAVKNFGTLDILINNAGLCGAPCPDIRNYSLSEFEMTFDVNVKGAFLSMKHAARVMIPEKKGSIVSLCSVGGVVGGVGP
HSYVGSKHAVLGLTRSVAAELGQHGIRVNCVSPYAVATKLALAHLPEEERTEDAFVGFRNFAAANANLKGVELTVDDVANAVLFL
ASDDSRYISGDNLMIDGGFTCTNHSFKVFR
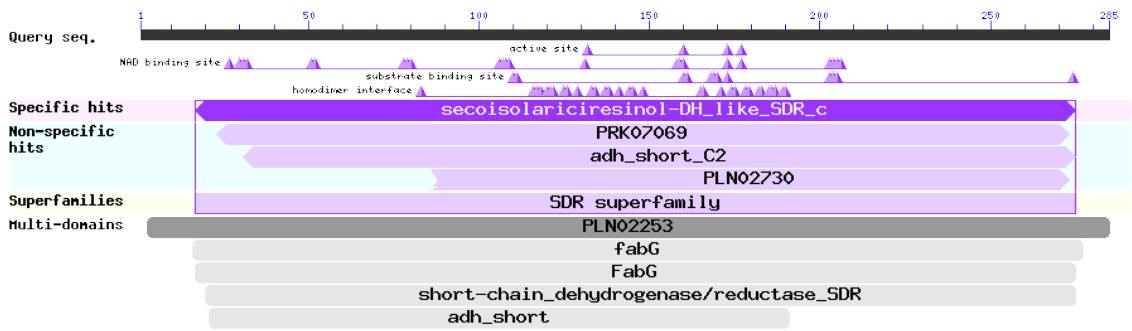
Name	Accession	Description	Interval	E-value
secoisolariciresinol-DH_like_SDR_c	cd05326	secoisolariciresinol dehydrogenase (secoisolariciresinol-DH)-like, classical (c) SDRs; ...	17-275	2.36e-136
PRK07069	PRK07069	short chain dehydrogenase; Validated	23-273	1.71e-38
adh_short_C2	pfam13561	Enoyl-(Acyl carrier protein) reductase;	31-275	5.74e-25
PLN02730	PLN02730	enoyl-[acyl-carrier-protein] reductase	86-273	1.23e-06
PLN02253	PLN02253	xanthoxin dehydrogenase	3-285	0e+00
fabG	PRK07231	3-ketoacyl-(acyl-carrier-protein) reductase; Provisional	16-277	2.15e-66
FabG	COG1028	Dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases) ...	17-275	1.52e-59
short-chain_dehydrogenase/reductase_SDR	TIGR03971	SDR family mycofactocin-dependent oxidoreductase; Members of this protein subfamily are ...	20-275	1.55e-54
adh_short	pfam00106	short chain dehydrogenase; This family contains a wide variety of dehydrogenases.	21-191	6.94e-20

```
PuXanDH    MSVPRLPPLAAAMARSAAAAASSSVPAFAATAGVPLGSVAARATASASTTAAVCRAGAGS

PpXanDH     MAPPPPDAAGVNPSAASRMDLTG--------------------------KRALVTG
PuXanDH    LFGTPLRGRAAAVAAAAAGRLPSTPSTSATTTTTIKTTTTTRGVPSMAADGSPKVAIVTG
AtXanDH      MSTNTESSSYSSLPSQRLLG--------------------------KVALITG

PpXanDH    GHRGIGAAIVLGLAAAGAHVAVIDRGGNASRTSDIPSRVRGYGVEFFAAAADLGDPEQVM
PuXanDH    ASRGIGKAIALALAAAGCKVVVN-YARSADAAASVVADIEAAGGEALAVRGDVSQAADVA
PyXanDH    ---------------------------------------AAKRDVCYPICFDPSNSEAVT
AtXanDH    GATGIGESIVRLFHKHGAKVCIVDLQDDLGGEVCKSLLRGESKETAFFIHGDVRVEDDIS
                                                              *        :

PpXanDH    AVVSRVLSEFGNIDILVNNAGITELQPLTTTPVHPDPPLSVLAWDAQFAVNVRAPMLLAQ
PuXanDH    ALFQTVVATYGAVDVLVNNAGITDTLLMR---------MKQAQWQEVIDLNLTGVFLCTQ
PyXanDH    AAVARIDADAWPVYVLINNSGILSNTLVEET--------SIDEWRNVFAQNVDSAFLLSR
AtXanDH    NAVDFAVKNFGTLDILINNAGLCGAPCPDIRN------YSLSEFEMTFDVNVKGAFLSMK
           .             : :*:**:*:            .   : :  *:. .:*  :

PpXanDH    AVAPAMVAARRGKIINVTSQAGTAA-LPLHAAYGASKAALDHLTRGMVAEWGRYNIQANA
PuXanDH    AAAKLMIKARAGRIVNIASVVGQIG-NPGQVNYAAAKGGVLGLTMATAKEVASRGVTVNA
PyXanDH    ALLPAMRKRKWGRIVNTCSLAGKTGGVTTGVAYSTTKGALQTLTFALARESAKDGITVNG
AtXanDH    HAARVMIPEKKGSIVSLCSVGGVVG-GVGPHSYVGSKHAVLGLTRSVAAELGQHGIRVNC
                  *   : ** :.  * *  .       *  :*  .:  **  .  *.   .: .*

PpXanDH    VAPTVIASDMGDAAWPPGAPKTVTMLDRIPAGRFGQAWEVADLVVFLAGGGAT-------
PuXanDH    VAPGFIASDMTAELPLDKIKAMIPMGRLGEAGRGGGHGHLPRHRPGRGVRDGAHV-----
PyXanDH    IAPAYIRTPTVETYPEDVVDMLLRYIPVSRFCEPEEFAHVVRFLISPMSGFITGEIIDQN
AtXanDH    VSPYAVATKLALAHLPEEERTEDAFVGFRNFAAANANLKGVELTVDDVANAVLFLASDDS
           ::*  : :                                           .
```

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PyXanDH        GGLHMS------------------
AtXanDH        RYISGDNLMIDGGFTCTNHSFKVFR
```

Figure S6. Bangiophycean XanDH homologues. Full-length cDNAs encoding XanDH homologues were found from *P. purpurea* and *P. umbilicalis*. For *P. yezoensis* XanDH (Py XanDH), two overlapping but not full-length ESTs, AV439406 and AV438237, were found in the *P. yezoensis* EST database by homology searches with the nucleotide sequence of *A. thaliana* XanDH (AtXanDH; accession no. NM_104113). The amino acid sequence deduced from these ESTs was designated PyXanDH. In addition, two ESTs, esContig7972 of *P. purpurea* and esContig5572 of *P. umbilicalis* were found by homology search at NoriBLAST with the AtXanDH sequence and designated PpXanDH and PuXanDH, respectively. Amino acid sequences and CD-search results of red algal XanDH homologues and AtXanDH, which were obtained using the NCBI Conserved Domain Database (CDD; www.ncbi.nlm.nih.gov/cdd), are represented. In addition, amino acid alignment obtained by CLUSTALW is shown, indicating the presence of the GXXXGXG residues for cofactor binding site and the YXXXK residues for catalytic activity (both are highlighted by red characters), both of which conserved in XanDH (Endo et al. 2014).

Reference: Endo A, Nelson KM, Thoms K, Abrams SR, Nambara E, Sato Y (2014) Functional characterization of xanthoxin dehydrogenase in rice. J Plant Physiol 171: 1231-1240

PpAA03-1

LQAAGEASYIDDLPMHKSGYFACLVTATVARGKLANIDASKALSAPGVGGFLSAADVPGDNNYNVTGGEKETIFAEETIDYFGQP
VGMILADSERHAREAALLVDVTYDAPEKAPVATIEQALAQPDTFVTPVRTEHKNGDVNEELEKAVCRTQGAVKVGTQKHFYMETQ
GAYALPDENGRIVVHSANQWPDGIQGAVARALGLPLAKTKIVFR
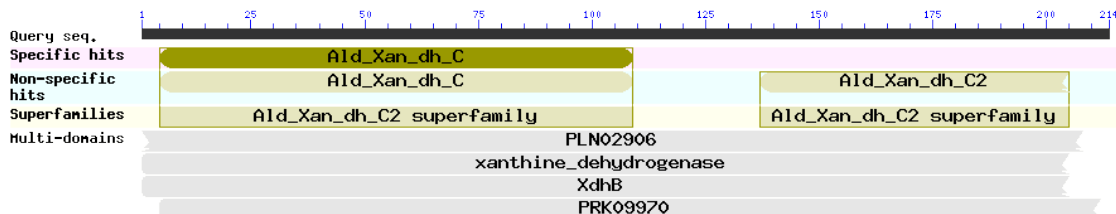
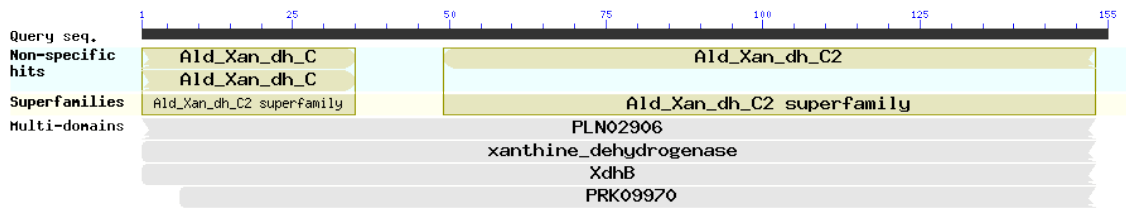| Name	Accession	Description	Interval	E-value
Ald_Xan_dh_C	pfam01315	Aldehyde oxidase and xanthine dehydrogenase, a/b hammerhead domain;	5-109	3.81e-29
Ald_Xan_dh_C	smart01008	Aldehyde oxidase and xanthine dehydrogenase, a/b hammerhead domain; Aldehyde oxidase catalyses ...	5-109	7.44e-27
Ald_Xan_dh_C2	pfam02738	Molybdopterin-binding domain of aldehyde dehydrogenase;	137-205	4.73e-14
PLN02906	PLN02906	xanthine dehydrogenase	1-208	1.37e-45
xanthine_dehydrogenase	TIGR02965	xanthine dehydrogenase, molybdopterin binding subunit;	1-205	8.19e-44
XdhB	COG4631	Xanthine dehydrogenase, molybdopterin-binding subunit B [Nucleotide transport and metabolism]	1-205	1.34e-42
PRK09970	PRK09970	xanthine dehydrogenase subunit XdhA; Provisional	5-212	1.65e-25

PpAA03-2

ARDQLVFYLNGKRVELNDADPEMTLIQFVRSIGLTGTKLGCAEGGCGACTVMISHYDTADRKVVHRSANGCLLPLCAVEGMAVTT
VEGLGNTKDGLNPVQERVAHSHGSQCGFCTPGIVMALYTFLRNHRAPRPRRSRRRWTATSAAARATGPSST
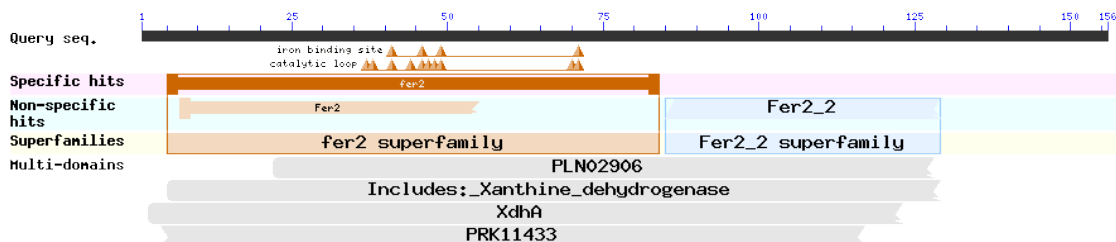
Name	Accession	Description	Interval	E-value
fer2	cd00207	2Fe-2S iron-sulfur cluster binding domain. Iron-sulfur proteins play an important role in ...	5-84	7.99e-08
Fer2_2	pfam01799	[2Fe-2S] binding domain;	85-129	1.74e-15
Fer2	pfam00111	2Fe-2S iron-sulfur cluster binding domain;	7-55	1.03e-06
PLN02906	PLN02906	xanthine dehydrogenase	22-128	6.54e-62
Includes:_Xanthine_dehydrogenase	TIGR02963	xanthine dehydrogenase, small subunit; Members of this protein family are the small subunit ...	5-129	4.02e-60
XdhA	COG4630	Xanthine dehydrogenase, iron-sulfur cluster and FAD-binding subunit A [Nucleotide transport ...	2-123	1.94e-40
PRK11433	PRK11433	aldehyde oxidoreductase 2Fe-2S subunit; Provisional	4-117	2.85e-21

PpAA03-3

VFAESSVEYCGQSVGLILADTRLHAEDAAKAVAVTCSNEKEPILSIEQALEAGSIFPDTITQGVAEYTQGDVSTGLANSDVTFSG
SIKAPMQYHFHMETHSCVVVPEENGKLRIHIGTQWPYLVQKVVARVLGWPEAKVHVGMKRAGGGYGAKIA

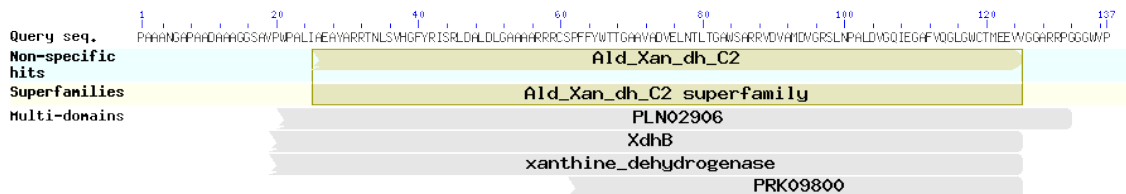| Name	Accession	Description	Interval	E-value
Ald_Xan_dh_C2	pfam02738	Molybdopterin-binding domain of aldehyde dehydrogenase;	49-153	1.26e-30
Ald_Xan_dh_C	smart01008	Aldehyde oxidase and xanthine dehydrogenase, a/b hammerhead domain; Aldehyde oxidase catalyses ...	1-35	7.71e-07
Ald_Xan_dh_C	pfam01315	Aldehyde oxidase and xanthine dehydrogenase, a/b hammerhead domain;	1-35	1.66e-06
PLN02906	PLN02906	xanthine dehydrogenase	1-153	9.34e-32
xanthine_dehydrogenase	TIGR02965	xanthine dehydrogenase, molybdopterin binding subunit; Members of the protein family are the ...	1-153	6.93e-30
XdhB	COG4631	Xanthine dehydrogenase, molybdopterin-binding subunit B [Nucleotide transport and metabolism]	1-153	3.04e-27
PRK09970	PRK09970	xanthine dehydrogenase subunit XdhA; Provisional	7-153	5.76e-21

PpAA03-4

NTGERVLVDISGVPELQARSVGPRGSLSVQPVSIDNFIALVNQARAAEKNEARVNNLTALLHHANKVANVHVRHAGSVAGNLVMA
KSLGFLSDLATILLGAEATVTLASRAGSRTVGMEEFLASPTFANGELVQSIAVHLASPDVRFSTYKAALRPQNSHAYINAALQVT
LGDNGIQKPVLAFGGVLPEDTAGSHAVRAKQTEEFLAG
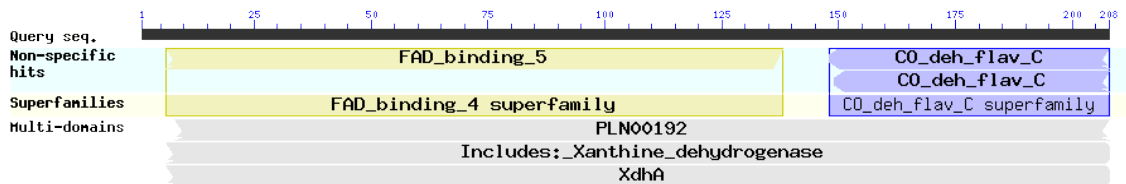
Name	Accession	Description	Interval	E-value
FAD_binding_5	pfam00941	FAD binding domain in molybdopterin dehydrogenase;	6-138	5.61e-18
CO_deh_flav_C	pfam03450	CO dehydrogenase flavoprotein C-terminal domain;	148-208	3.02e-07
CO_deh_flav_C	smart01092	CO dehydrogenase flavoprotein C-terminal domain;	149-208	5.29e-05
PLN00192	PLN00192	aldehyde oxidase	8-208	7.90e-31
Includes:_Xanthine_dehydrogenase	TIGR02963	xanthine dehydrogenase, small subunit; Members of this protein family are the small subunit ...	6-208	1.08e-15
XdhA	COG4630	Xanthine dehydrogenase, iron-sulfur cluster and FAD-binding subunit A [Nucleotide transport ...	6-208	3.86e-13

PpAA03-5

PAAANGAPAADAAAGGSAVPWPALIAEAYARRTNLSVHGFYRISRLDALDLGAAAARRRCSPFFYWTTGAAVADVELNTLTGAWS
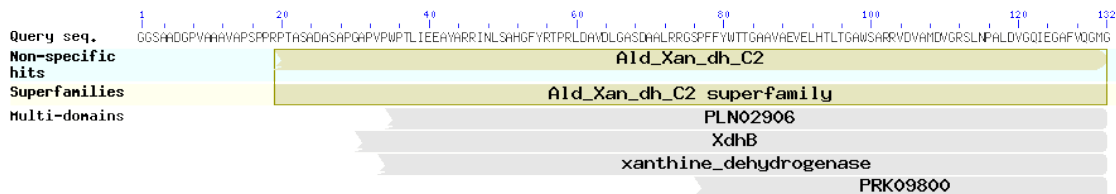ARRVDVAMDVGRSLNPALDVGQIEGAFVQGLGWCTMEEVVGGARRPGGGWVP

Name	Accession	Description	Interval	E-value
Ald_Xan_dh_C2	pfam02738	Molybdopterin-binding domain of aldehyde dehydrogenase;	25-125	1.17e-25
PLN02906	PLN02906	xanthine dehydrogenase	20-132	5.03e-34
XdhB	COG4631	Xanthine dehydrogenase, molybdopterin-binding subunit B [Nucleotide transport and metabolism]	19-125	1.03e-32

xanthine_dehydrogenase	TIGR02965	xanthine dehydrogenase, molybdopterin binding subunit; Members of the protein family are the ...	19-125	1.55e-32
PRK09800	PRK09800	putative hypoxanthine oxidase; Provisional	61-125	1.22e-05

PuAAO3

GGSAADGPVAAAVAPSPPRPTASADASAPGAPVPWPTLIEEAYARRINLSAHGFYRTPRLDAVDLGASDAALRRGSPFFYWTTGA
AVAEVELHTLTGAWSARRVDVAMDVGRSLNPALDVGQIEGAFVQGMG

Name	Accession	Description	Interval	E-value
Ald_Xan_dh_C2	pfam02738	Molybdopterin-binding domain of aldehyde dehydrogenase;	19-132	3.07e-26
PLN02906	PLN02906	xanthine dehydrogenase	34-132	5.31e-35
XdhB	COG4631	Xanthine dehydrogenase, molybdopterin-binding subunit B [Nucleotide transport and metabolism]	30-132	7.89e-34
xanthine_dehydrogenase	TIGR02965	xanthine dehydrogenase, molybdopterin binding subunit; Members of the protein family are the ...	33-132	1.68e-33
PRK09800	PRK09800	putative hypoxanthine oxidase; Provisional	76-132	1.05e-03

AtAAO3

MDLEFAVNGERFKIDSVDPSTTLLEFLRLNTPFKSVKLGCGEGGCGACLVVLSKYDPELDQVKECCINSCLTLLCSVNGCSITTS
EGLGNTKKGFHPIHKRFAGFHASQCGFCTPGMCISLYSSLANAENNSSKDFTVSEAEKSVSGNLCRCTGYRPIVDACKSFASDVD
IEDLGLNSFWKKGESKEVMFKNLPPYNPKDHLVTFPEFLKKKEKVDNGSDHLKYRWTTPFSVAELHNIMEAANSGDSLKLVVGNT
GTGYYKDEERFDRYIDISNIPEMSMIKKDEKGIEIGAAVTISNAIDALEKESKSSYVFKKMATHMEKIGNRSIRNSGSIGGNLVM
AQSRKFPSDVTTLLLAVDASVYMLNGRKTEKVTLQEFLELSPVLDSKRVLLKVEIPSWTAPSGDDTEFLFESYRAAPRSIGNALP
YLNAAFLALVSRQEASRKGVTVEKCFLAFGSYGGDHSIRAIEVETFLTGKLLSYSVLYEAVGLLKGIIVPGKDTLHSEYRKSLAV
GYLFEFFYPLIESGHRICSLDSGNKHNNSHVDTVKSLPFLSSSQQVLESNEFKPIGEAVIKVGAALQASGEAVFVDDIPTLPDCL
HGAFIYSTEPLAKIKSLSFRENVTPTGVFAVLTFKDIPQQGQNIGSKTLFGPGPLFADELTRCAGQRIALVVADTQKHADMAAKL
AVVEYDTKNLEQPILTVEDAVKRSSFFEVHPMFYPEPVGDVIKGMEEAERKIISSELRLGSQYFFYMEPQTALALPDEDNCVKVF
SSSQAPEYVHSVIATCLGIQEHNVRVITRRVGGGFGGKAVKSMPVATACALGAYKLQRPVKMFLNRKTDMIMAGGRHPMKINYNV
GFRSDGKLTALELTMLIDAGLEPDVSPIMPRNIMGPLRKYDWGALSFDVKVCKTNCLSRTAMRAPGEVQGSYIAESIIENVASSL
QMDVDAVRKINLHTYDSLRKFYNHIAGDPDEYTLPLLWEKLEISSKFKERSEMVKEFNLCNVWRKRGISRVPIVHQVMQRPTPGK
VSILSDGSVVVEVGGIEIGQGLWTKVQQMVAYGLGMVKCEGNEKLLDRIRVVQSDTLGMIQGGFTAGSTTSESSCEAVRLCCVIL
VERLKPIMDQMMMEKSGSVTWNILIQQAYGQYINLSASTLYKPEYSSMEYLNYGVGVSEVEVDLVTGKTEILRSDIIYDCGKSLN
PAVDLGQTEGAFVQGIGFFMMEEYTTDEKGLVVQQGTWDYKIPTVDTIPKHFNVEIVNTGHHKNRVLSSKASGEPPLLLAASVHC
ATRSAIREARKHSLSSNFIDGSDSEFELPVPATMPVVKSLCGLYSVEKYLQGKIKGQ
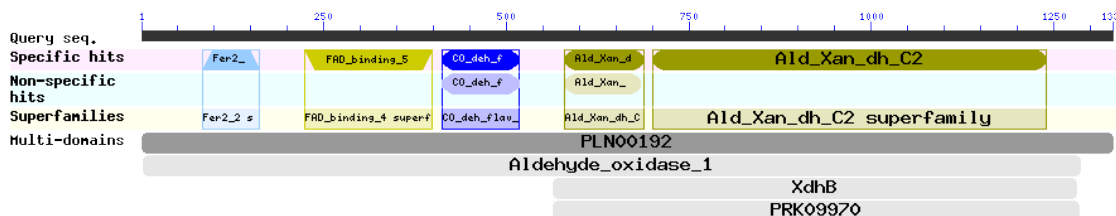
Name	Accession	Description	Interval	E-value
Ald_Xan_dh_C2	pfam02738	Molybdopterin-binding domain of aldehyde dehydrogenase;	700-1239	0e+00
FAD_binding_5	pfam00941	FAD binding domain in molybdopterin dehydrogenase;	223-398	3.04e-46
Ald_Xan_dh_C	pfam01315	Aldehyde oxidase and xanthine dehydrogenase, a/b hammerhead domain;	579-689	1.01e-39

Ald_Xan_dh_C	smart01008	Aldehyde oxidase and xanthine dehydrogenase, a/b hammerhead domain; Aldehyde oxidase catalyses ...	579-686	3.18e-28
Fer2_2	pfam01799	[2Fe-2S] binding domain;	84-161	5.00e-23
CO_deh_flav_C	pfam03450	CO dehydrogenase flavoprotein C-terminal domain;	412-517	3.60e-17
CO_deh_flav_C	smart01092	CO dehydrogenase flavoprotein C-terminal domain;	412-517	3.65e-08
PLN00192	PLN00192	aldehyde oxidase	1-1331	0e+00
Aldehyde_oxidase_1	TIGR02969	aldehyde oxidase; Members of this family are mammalian aldehyde oxidase (EC 1.2.3.1) isozymes, ...	2-1286	0e+00
XdhB	COG4631	Xanthine dehydrogenase, molybdopterin-binding subunit B [Nucleotide transport and metabolism]	564-1281	2.43e-152
PRK09970	PRK09970	xanthine dehydrogenase subunit XdhA; Provisional	565-1284	1.82e-67

Figure S7. Bangiophycean AAO3 homologues. By homology search at NoriBLAST
with AAO3 sequence from *A. thaliana* (AtAAO3; accession no. Q7G9P4), an EST,
esFYYDJ9L01CO4XC, from *P. umbilicalis* and five ESTs, esisotig09565,
esGAQG33Y01DZ5D6, esGAQG33Y02ILR75, esisotig09716 and esisotig12382, from
P. purpurea were obtained and designated PuAAO3 and PpAAO3-1 to PpAAO3-5,
respectively. Amino acid sequences and CD-search results of red algal AAO3
homologues and AtAAO3, which were obtained using the NCBI Conserved Domain
Database (CDD; www.ncbi.nlm.nih.gov/cdd), are represented. All Bangiophycean
AAO3 homologues are non-full-length and very short, which prevent good alignment
with CLUSTALW.