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Research Article

Differential gene expression in *Pyropia columbina* (Bangiales, Rhodophyta) under natural hydration and desiccation conditions

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ABSTRACT. In rocky shores, desiccation is triggered by daily tide changes, and experimental evidence suggests that local distribution of algal species across the intertidal rocky zone is related to their capacity to tolerate desiccation. In this context, the permanence of *Pyropia columbina* in the high intertidal rocky zone is explained by its exceptional physiological tolerance to desiccation. This study explored the metabolic pathways involved in tolerance to desiccation in the Chilean *P. columbina*, by characterizing its transcriptome under contrasting conditions of hydration. We obtained 1,410 ESTs from two subtracted cDNA libraries in naturally hydrated and desiccated fronds. Results indicate that transcriptome from both libraries contain transcripts from diverse metabolic pathways related to tolerance. Among the transcripts differentially expressed, 15% appears involved in protein synthesis, processing and degradation, 14.4% are related to photosynthesis and chloroplast, 13.1% to respiration and mitochondrial function (NADH dehydrogenase and cytochrome c oxidase proteins), 10.6% to cell wall metabolism, and 7.5% are involved in antioxidant activity, chaperone and defense factors (catalase, thioredoxin, heat shock proteins, cytochrome P450). Both libraries highlight the presence of genes/proteins never described before in algae. This information provides the first molecular work regarding desiccation tolerance in *P. columbina*, and helps, to some extent, explaining the classical patterns of ecological distribution described for algae across the intertidal zone.

Keywords: *Pyropia*, desiccation stress, ESTs, seaweeds, transcriptomics, proteins.

Expresión diferencial de genes en *Pyropia columbina* (Bangiales, Rhodophyta) bajo hidratación y desecación natural

RESUMEN. En zonas rocosas costeras, la desecación es gatillada por cambios diarios en los niveles de marea, y la evidencia experimental indica que la distribución de las algas en la zona intermareal está relacionada con su capacidad para tolerar la desecación. En este contexto, la presencia de *Pyropia columbina* en la zona alta del intermareal se explica por su excepcional tolerancia fisiológica a la desecación. Este estudio explora las vías metabólicas involucradas en la tolerancia a la desecación en *P. columbina*, a través de la caracterización de su transcriptoma bajo condiciones de hidratación contrastantes. Se obtuvo 1,410 ESTs provenientes de dos librerías de substracción de cDNA de frondas naturalmente hidratadas y desecadas. Los transcriptomas de ambas librerías contienen transcritos de diversas rutas metabólicas relacionadas a la tolerancia. Entre los transcritos expresados 15% están involucrados en la síntesis de proteínas, su procesamiento y degradación, 14,4% asociados a fotosíntesis y cloroplasto, 13,1% a respiración y función mitocondrial, 10,6% al metabolismo de la pared celular y 7,5% a la actividad antioxidante, proteínas chaperonas y factores de defensa (catalasa, tiorredoxina, proteínas de shock térmico, citocromo P450). En ambas librerías se destaca la presencia de genes/proteínas no descritos en algas. Esta información proporciona

el primer trabajo molecular que estudia la tolerancia a desecación en *P. columbina* y sus resultados ayudan a explicar los patrones clásicos de distribución descritos para algas en la zona intermareal.

Palabras clave: *Pyropia*, estrés por desecación, ESTs, macroalgas, transcriptómica, proteínas.

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INTRODUCTION

Red algae (Rhodophyta), the most ancient lineage of photosynthetic eukaryotes (Baldauf *et al.*, 2000; Yoon *et al.*, 2004), are distributed worldwide and include several commercially important species. *Porphyra* and *Pyropia* spp. are rhodophytes that represent an annual value of over US\$1.3 billion (Blouin *et al.*, 2011). In Chile, *Pyropia columbina* (Montagne) W.A. Nelson (formerly *Porphyra columbina* (Sutherland *et al.*, 2011) is one of the economically important species, together with members of the rhodophycean genera *Mazzaella*, *Gracilaria* and *Gelidium* (Santelices, 1989; Hoffmann & Santelices, 1997; Buschmann *et al.*, 2008), and it is found along the Chilean coast from 20° to 54°S (Hoffmann & Santelices, 1997; Guiry & Guiry, 2013). This species has a biphasic life history that includes a microscopic sporophyte generation (2n, *conchocelis* stage) alternating with a macroscopic generation of male and female gametophytes (n). The foliose gametophytes constitute the edible “Nori”.

Water, and its intracellular balance, is a critical factor for all living organisms in both terrestrial and marine ecosystems. Mobile animals actively avoid desiccation, induced by water deficiency, while other organisms, such as resurrection plants—a small group of angiosperms that live in the most arid habitats of the world—are adapted to tolerate water losses of up to 90% (Gaff, 1987). This adaptation, in general terms, is based on the ability of an organism to equilibrate its internal water potential with the dry environment, and re-start normal functions when re-hydrated (Alpert, 2000). Several studies using resurrection plants as model have been conducted to fully understand their impressive adaptation to desiccation (Scott, 2000). Recent advances in our understanding of the mechanisms of tolerance in these organisms have revealed changes at the morphological level, osmolites and protein synthesis, and a decline in ROS (reactive oxygen species) production and photosynthesis rate (Ingram & Bartels, 1996; Hoekstra *et al.*, 2001; Bernacchia & Furini, 2004; Vicré *et al.*, 2004; Dinakar *et al.*, 2012). More specifically, it has been observed that the plant hormone ABA is accumulated in desiccated resurrection plants, which

induces the expression of several proteins related to desiccation (Bartels *et al.*, 1990; Dinakar *et al.*, 2012). Also, leaves of resurrection plants tend to curl to reduce water loss and minimize oxidative damage due to desiccation (Vicré *et al.*, 2004; Farrant *et al.*, 2007; Toldi *et al.*, 2009). Oxidative damage is attenuated or avoided by increasing antioxidant activity of some enzymes (*e.g.*, ascorbate peroxidase, glutathione reductase, superoxide dismutase, among others) and levels of antioxidant compounds (*e.g.*, anthocyanins) (Farrant *et al.*, 2007; Toldi *et al.*, 2009; Dinakar *et al.*, 2012). Additionally, these plants reduce photosynthetic activity to minimize photo-oxidative damage that could lead to increased ROS levels. Thus, it seems clear that diverse metabolic pathways are involved in attenuating the oxidative stress condition caused by desiccation.

In the Chilean coastal ecosystems, *P. columbina* grows abundantly along the upper intertidal zone (Alveal, 1970; Santelices, 1989; Hoffmann & Santelices, 1997), where it is exposed to a wide range of environmentally stressful conditions, mainly desiccation-driven stress induced by low tides and air exposure (Contreras-Porcia *et al.*, 2011a). *P. columbina* is well adapted to daily extremes, which range from exposure to water (full hydration) during high tides to long exposure to air (maximum desiccation) during low tides. Natural exposure to these extreme regimes have been described in other organisms exposed to desiccation. It is already known that *Porphyra* and *Pyropia* species have high tolerance to desiccation, and quickly recover photosynthetic activity once rehydrated after a period of desiccation (*e.g.*, Smith & Berry, 1986; Kim *et al.*, 2008; Contreras-Porcia *et al.*, 2011a; Gao & Wang, 2012). Basic physiology of these organisms, including the mechanisms to tolerate environmentally stressful conditions, remains poorly studied. However, it is known that, in *P. columbina*, desiccation induces losses *ca.* 96% of the water content and enhanced significantly the production of ROS (Contreras-Porcia *et al.*, 2011a). The quick return of ROS to their basal levels during high tide is explained by an efficient activation of the antioxidant system. In comparison, species inhabiting the lower intertidal zone [*e.g.*,

Mazzaella laminarioides (Bory de Saint-Vincent) Fredericq, *Ulva compressa* Linnaeus and *Lessonia spicata* (Suhr) Santelices] are more sensitive to desiccation, and this seems related to the absence of efficient mechanisms to attenuate the over-production of ROS during rehydration (Contreras-Porcia *et al.*, 2012; López-Cristoffanini *et al.*, 2013). Flores-Molina *et al.* (unpublished data) recently provided some physiological and biochemical bases that help explain the role of desiccation on species distribution across the intertidal zone. They reported that sensitive species displayed i) inactivation of antioxidant enzymes, ii) over-oxidation of biomolecules, and iii) inactivation of the photosystem II. For example, *Ulva compressa* (Chlorophyta, Plantae) and *Scytosiphon lomentaria* (Lyngbye) Link (Ochrophyta, Chromista) inhabiting the mid-intertidal zone have lower tolerance to desiccation than *P. columbina*. However, they tolerate desiccation better than *Lessonia spicata* (Ochrophyta, Chromista) and *Gelidium rex* Santelices & I.A. Abbott (Rhodophyta, Plantae), both lower intertidal species.

Given the ecological and economic relevance of *Pyropia* and that some basic biochemical and physiological information on the mechanisms involved in tolerance to desiccation is known, we focused this work in determining the genes/proteins that are differentially expressed in *P. columbina* during the hydration-desiccation cycle, using Suppression Subtractive Hybridization (SSH) and ESTs determined by pyrosequencing (454 Life Sciences, Roche). The results of this study will help to elucidate the genetic basis underlying the high tolerance to desiccation displayed by this species, and will broaden our knowledge of the molecular biology/ecology of this organism and other macroalgal species.

MATERIALS AND METHODS

Ethics statement

No specific permits were required for the described field studies. The study area is unrestricted to public access and use, and is not privately owned or designated as a protected area (reserves or parks). No protected or endangered species were involved in this study. Fronds of *P. columbina* were collected along 200-300 m of coastline during high (naturally hydrated plants, 100% relative water content (RWC)) and low tide (naturally desiccated plants, ca. 4% RWC) in Maitencillo beach, Valparaíso (32°39.5'S, 71°26.6'W). After collection, fronds were quickly rinsed (15-20 s) in 0.22 µm-filtered seawater,

manually cleaned and frozen on site with liquid nitrogen.

RNA extraction

Total RNA was isolated from 20-30 g of fresh tissue of naturally hydrated and desiccated, pooled fronds of *P. columbina*. Tissue, frozen in liquid nitrogen, was homogenized in 25 mL of lysis buffer containing 4 M guanidinium thiocyanate, 25 mM EDTA, 200 mM sodium acetate, 2% polyvinylpyrrolidone (PVP-40) and 1% 2-mercaptoethanol. The homogenate was incubated for 10 min at 70°C with constant agitation in the presence of 20% sarcosin, and then centrifuged for 5 min at 16000 g. The RNA present in the supernatant solution was purified and re-extracted using an RNeasy mini Kit (Qiagen, Hilden, Germany), according to the manufacturer protocol. RNA quality and yield was assessed by spectrophotometry (NanoDrop™ 1000 Spectrophotometer, Thermo Scientific, DE, USA) and denaturing agarose gel electrophoresis. Finally, the mRNA was obtained from the total RNA extracted (ca. 290-300 µg) using DynaBeads (Invitrogen, Oregon, USA). Prior to RNA extraction, all material was treated in 0.1% DEPC water.

Preparation of the cDNA libraries by SSH and next-generation sequencing

The synthesis of cDNAs, from both natural conditions (*i.e.*, natural hydration and desiccation stress), were obtained using a SMART™ cDNA Library Construction Kit (Clontech, Mountain View, CA, USA), as described in Wellenreuther *et al.* (2004). Then, two subtracted cDNA libraries were constructed: one with genes expressed exclusively under hydration (UH) and the other by those expressed under desiccation stress (UD), as described in Diatchenko *et al.* (1996). cDNAs were then sequenced by 454-pyrosequencing (Margulies *et al.*, 2005). cDNAs were ligated to 454 self-made adaptors with Multiplex Identifier Adaptors (MIDs) for the GS FLX Titanium Chemistry following Roche's technical bulletin TCB 09004 introducing *Sfi*I-sites. The 454 libraries were immobilized on beads and clonally amplified using a "GS FLX Titanium LV emPCR Kit". The libraries were then sequenced using a "GS FLX Titanium Sequencing Kit XLR70" and a "GS FLX Titanium PicoTiterPlate Kit". All kits were purchased from Roche and used according to the manufacturer protocols.

Data assembly and bioinformatics analysis

Readings from both libraries were processed by self-written Perl scripts and assembled into coatings, *i.e.*,

representing putative transcripts using MIRA 3 assembly (Contreras-Porcia *et al.*, 2011b). The expressed sequence tags (ESTs) determined from both libraries were subjected to separated bioinformatics analyses. ESTs were analyzed for sequence similarities using the BLASTX program (NCBI, MD, USA). Reading frames with the highest sequence similarity scores were used to analyze protein identity using the BLASTP program. Threshold values were set above 50 for high-scoring segment pairs, with a minimum significance at least of $e \leq 10^{-4}$ and an identity higher than 30%. ESTs coding for known proteins were classified into functional categories by the KO (KEGG Orthology) database for ortholog grouping and hierarchical classification of genes, according their functionality (Kanehisa *et al.*, 2004) by using the BLAST2GO software (Götz *et al.*, 2008). Finally, the putative subcellular localization of the proteins was determined using the TARGETP (<http://www.cbs.dtu.dk/services/TargetP> Emanuelsson *et al.*, 2000), WOLFPSORT (<http://psort.ims.u-tokyo.ac.jp>), ChloroP (<http://www.cbs.dtu.dk/services/ChloroP> Emanuelsson *et al.*, 1999) and PSORTb (<http://www.psort.org/psortb> Yu *et al.*, 2010) servers.

RESULTS

EST sequencing and assembly

A total of 8,054 sequence reads were obtained from the library enriched with hydration-responsive exclusive transcripts (UH library, Table 1). Moreover, 8,432 sequence reads were obtained from the library enriched with desiccation-responsive exclusive transcripts (UD library). In the UH library, 49.2% of ESTs ranged from 200 to 500 bp, with an average size of 423 bp. In the UD library, 46% of the ESTs ranged from 200 to 500 bp, with an average size of 385 bp. The rest of the transcripts in both libraries ranged between 100 to 190 bp. The sequences of both libraries are available on the EMBL Nucleotide Sequence Database (<http://www.ebi.ac.uk/embl>) with accession numbers HE858615 to HE859412 for UH and HE859413 to HE859937 for UD libraries.

Gene ontology from naturally hydrated and desiccated *P. columbina* transcriptome

Almost 59% of the total ESTs (*i.e.*, 491) from UH displayed no similarity with sequences available in databases; whereas the remaining 41% (*i.e.*, 347) were similar to registered proteins (Table 1). Similarities (68.8% of them) concentrated in sequences reported for Arthropoda (*e.g.*, *Culex quinquefasciatus*), Chordata (*e.g.* *Danio rerio*) and Mollusca (*e.g.*, *Littorina saxatilis*). Another 18% of the total ESTs

were similar to proteins from the kingdom Plantae, mainly Rhodophyta (*e.g.*, *Porphyra purpurea*) and Tracheophyta (*e.g.*, *Vigna unguiculata*), and 10.7% to the kingdom Bacteria, mainly Proteobacteria (*e.g.*, *Haemophilus influenzae*) and Cyanobacteria (*e.g.*, *Microcystis aeruginosa*). Only 2.5% of the sequences were similar to proteins from Protozoa (*i.e.*, *Dictyostelium discoideum*), Fungi (*i.e.*, *Saccharomyces cerevisiae*) and Chromista (*i.e.*, *Pylaiella littoralis*). Finally, analysis of the amino terminal sequences showed that 51.8% of the identified proteins were potentially assignable to the cytosol, 28.6% to mitochondria, 13% to the chloroplast, and 4.4% to the nucleus (Table 1).

In the UD library, 72% of the total ESTs (*i.e.*, 412) displayed no similarity with previously reported sequences (Table 2). Of the remaining 28% (*i.e.*, 160 ESTs) showing similarity with proteins registered in databases: 37% were similar to those reported for Plantae, mainly Rhodophyta (*e.g.*, *Porphyra purpurea*) and Tracheophyta (*e.g.*, *Arabidopsis thaliana*), 29.4% with proteins from Bacteria, mainly Proteobacteria (*e.g.*, *Burkholderia multivorans*) and Cyanobacteria (*e.g.*, *Thermosynechococcus elongatus*), 21% with proteins from Animalia, mainly Arthropoda (*e.g.*, *Aedes aegypti*), Chordata (*e.g.*, *Mauremys mutica*) and Mollusca (*e.g.*, *Crassostrea virginica*), and 7.5% with proteins from Chromista, mainly Ochrophyta (*e.g.*, *Ectocarpus siliculosus*). Only 5% of the sequences were similar to proteins from Protozoa (*i.e.*, *Dictyostelium discoideum*) and Fungi (*i.e.*, *Leptosphaeria maculans*). Finally, analysis of the amino terminal sequences showed that 47% of the identified proteins were potentially assignable to cytosol, 21-23% to both mitochondria and chloroplast, and 7% to the nucleus (Table 2).

Functional categorization

The identified genes/proteins were classified into thirteen functional categories according their functionality (Fig. 1). For example, most ESTs from hydrated fronds (Table 1) matched with proteins involved in protein synthesis, processing and degradation (*ca.* 24%, peptidylprolyl cis-trans isomerases, ubiquitin and proteasome proteins), respiration and mitochondria (*ca.* 14%), antioxidant function, chaperone and defense factors (*ca.* 9%, *e.g.*, peroxiredoxin (PRX), arachidonate 5-lipoxygenase, a glutathione S-transferase, and several cytochrome P450 and HSPs), cell motility (*ca.* 9%) and basal metabolism (*ca.* 8%; *e.g.*, glyceraldehyde 3-phosphate dehydrogenase and L-lactate dehydrogenase). However, the transcriptome induced by desiccation (Fig. 1)

Table 1. Functional category, identity and potential cellular destination of proteins, and accession number of identified ESTs in *P. columbina* under natural hydration (UH library). *E* value: the best (lowest) Expect value (*E* value) of all alignments from that database sequence, D*: Putative destination.

Functional category	Putative Identity	Species and accession number	<i>E</i> value	D*	<i>Pyropia</i> accession number
1. Signal transduction	Phosphatidylinositol 3-kinase 1	<i>Culex quinquefasciatus</i> / XP_001847784.1	4.00E-07	nuc	HE859298
	MAP kinase activated protein-kinase-2	<i>Glossina morsitans morsitans</i> / ABC25082.1	2.00E-47	cyt	HE859158
	Low-density lipoprotein receptor-related protein 1B-like	<i>Xenopus (Silurana) tropicalis</i> / XP_002937402.1	5.00E-14	cyt	HE858680
	Low-density lipoprotein receptor-related protein 2 precursor	<i>Danio rerio</i> / NP_001181916.1	2.00E-19	cyt	HE858962
	Mitogen-activated protein kinase kinase kinase 15	<i>Apis mellifera</i> / XP_003250315.1	4.00E-19	cyt	HE859035
	Neurochondrin	<i>Drosophila melanogaster</i> / NP_649658.1	4.00E-20	n.d.	HE859071
	Neurochondrin-like protein	<i>Harpegnathos saltator</i> / EFN88263.1	2.00E-16	n.d.	HE859267
	Serine/threonine kinase	<i>Culex quinquefasciatus</i> / XP_001844678.1	6.00E-33	nuc	HE859315
	Protein serine/threonine kinase, putative	<i>Aedes aegypti</i> / XP_001660772.1	2.00E-21	nuc	HE858859
	Ras-related protein, isoform B	<i>Drosophila melanogaster</i> / NP_726881.1	4.00E-07	nuc	HE859339
	Serine/threonine kinase receptor-associated protein	<i>Glossina morsitans morsitans</i> / ADD19360.1	3.00E-70	nuc	HE858825
	Serine/threonine protein kinase	<i>Aedes aegypti</i> / XP_001655486.1	5.00E-44	cyt	HE859285
	Serine/threonine-protein phosphatase 5	<i>Culex quinquefasciatus</i> / XP_001850926.1	4.00E-45	cyt	HE859130
	SNF4/AMP-activated protein kinase gamma subunit, isoform M	<i>Drosophila melanogaster</i> / NP_001097854.1	4.00E-26	cyt	HE858743
	Troponin C type IIIa	<i>Apis mellifera</i> / NP_001011651.1	9.00E-21	mit	HE859041
	Calmodulin	<i>Pyropia yezoensis</i> / ABN41559.1	2.00E-52	cyt	HE858656
	Cyclic AMP phosphoprotein	<i>Scophthalmus maximus</i> / ABJ98640.1	1.00E-10	cyt	HE858976
	GAF sensor signal transduction histidine kinase	<i>Microcoleus vaginatus</i> FGP-2 / ZP_08492202.1	5.00E-18	cyt	HE858774
	Guanine nucleotide binding protein beta polypeptide 2- like 1	<i>Lethenteron camtschaticum</i> / BAE93065.1	9.00E-109	cyt	HE858703
	Putative guanine nucleotide binding protein beta polypeptide 2-like 1 protein	<i>Ovis aries</i> / ABY75292.1	2.00E-43	cyt	HE858908
	Troponin T	<i>Pediculus humanus corporis</i> / XP_002424248.1	3.00E-31	cyt	HE859343
	Calreticulin	<i>Eisenia andrei</i> / ABI74618.1	6.00E-42	mit	HE859085
	Adenylyl cyclase associated protein	<i>Gossypium herbaceum</i> subsp. <i>africanum</i> / ADZ16114.1	2.00E-22	cyt	HE858826
	Transcription factor BTF3 homolog 4	<i>Salmo salar</i> / ACI69109.1	5.00E-45	nuc	HE858889
2. Transcription, splicing, and replication	Transcription factor	<i>Brugia malayi</i> / XP_001891758.1	4.00E-26	nuc	HE858778
	TFIIH basal transcription factor complex P44 subunit	<i>Culex quinquefasciatus</i> / XP_001845822.1	2.00E-07	nuc	HE858942
	RNA polymerase beta subunit	<i>Pyropia yezoensis</i> / YP_536931.1	1.00E-21	chl	HE858844
	Homeotic protein spalt-major	<i>Camponotus floridanus</i> / EFN74164.1	3.00E-12	n.d.	HE859115
	Nascent polypeptide associated complex protein alpha subunit	<i>Glossina morsitans morsitans</i> / ADD19571.1	8.00E-17	n.d.	HE858969
	Similar to nuclear histone binding protein	<i>Strongylocentrotus purpuratus</i> / XP_791433.2	1.00E-11	nuc	HE858932
	orf544 reverse transcriptase	<i>Porphyra purpurea</i> / NP_049292.1	6.00E-47	mit	HE858715
	Reverse transcriptase all	<i>Saccharomyces cerevisiae</i> S288c / NP_009310.1	1.00E-12	mit	HE858909
	Reverse transcriptase homolog	<i>Pylaiella littoralis</i> / BAG06161.1	2.00E-05	mit	HE858919
	RNA-directed DNA polymerase	<i>Moorea producens</i> 3L / ZP_08427361.1	6.00E-05	mit	HE859330

Continuation

Functional category	Putative Identity	Species and accession number	E value	D*	Pyropia accession number
	RNA-directed DNA polymerase	<i>Microcystis aeruginosa</i> NIES-843 / YP_001659893.1	3.00E-19	mit	HE859084
	RNA-directed DNA polymerase (reverse transcriptase)	<i>Microcoleus vaginatus</i> FGP-2 / ZP_08493544.1	7.00E-05	mit	HE859136
	RNA-directed DNA polymerase (reverse transcriptase)	<i>Arthrospira maxima</i> CS-328 / ZP_03271771.1	2.00E-11	mit	HE858655
	Retrotransposon protein	<i>Oryza sativa Indica Group</i> / ABR26094.1	5.00E-14	nuc	HE858617
	Retrotransposon protein	<i>Oryza sativa Indica Group</i> / ABR26094.1	3.00E-09	mit	HE859408
	RNA-binding protein 8A	<i>Apis mellifera</i> / XP_395245.2	2.00E-35	cyt	HE859164
	rRNA intron-encoded homing endonuclease	<i>Thalassiosira pseudonana</i> CCMP1335 / XP_002294430.1	8.00E-12	mit	HE858662
	Ribonuclease R	<i>Weeksella virosa</i> DSM 16922 / YP_004238302.1	3.00E-23	cyt	HE858827
	rRNA promoter binding protein	<i>Brugia malayi</i> / XP_001891797.1	7.00E-05	mit	HE859073
	ATP-dependent helicase	<i>Zobellia galactanivorans</i> / CAZ98388.1	5.00E-14	n.d.	HE859151
	rRNA intron-encoded homing endonuclease	<i>Medicago truncatula</i> / XP_003614389.1	2.00E-05	cyt	HE858668
	Metal-dependent RNase	<i>Streptococcus pyogenes</i> M49 591 / ZP_00366321.1	2.00E-20	cyt	HE858664
3. Basal metabolism	Mitochondrial Malate dehydrogenase 2	<i>Culex quinquefasciatus</i> / XP_001849862.1	2.00E-26	mit	HE858747
	Glyceraldehyde 3-phosphate dehydrogenase	<i>Simulium nigritanum</i> / ACZ28396.1	4.00E-05	cyt	HE858837
	Glyceraldehyde 3-phosphate dehydrogenase	<i>Tribolium castaneum</i> / XP_974181.1	5.00E-32	cyt	HE858850
	Pyruvate dehydrogenase	<i>Aedes aegypti</i> / XP_001648922.1	3.00E-94	mit	HE859341
	Medium chain acyl-coenzyme A dehydrogenase	<i>Pachycara brachycephalum</i> / AEC32921.1	3.00E-34	mit	HE859282
	L-lactate dehydrogenase-like	<i>Apis mellifera</i> / XP_394662.4	3.00E-37	cyt	HE858775
	Acetyl-CoA carboxylase beta subunit	<i>Porphyrha purpurea</i> / NP_053808.1	7.00E-33	chl	HE859176
	NAD-dependent epimerase/dehydratase	<i>Zea mays</i> / NP_001148959.1	1.00E-13	cyt	HE859024
	Phosphogluconate dehydrogenase	<i>Tipula abdominalis</i> / ACH95392.1	5.00E-35	cyt	HE859302
	Phosphoglycerate mutase	<i>Porphyrha purpurea</i> / NP_053989.1	7.00E-34	chl	HE858842
	Mitochondrial Succinyl-CoA ligase [ADP-forming] subunit beta	<i>Camponotus floridanus</i> / EFN67099.1	5.00E-43	mit	HE859064
	Succinyl-CoA ligase [GDP-forming] subunit alpha	<i>Anolis carolinensis</i> / XP_003221906.1	2.00E-10	mit	HE858963
	Transcript antisense to ribosomal RNA protein 1(Tar1p)	<i>Saccharomyces cerevisiae</i> S288c / NP_690845.1	7.00E-06	mit	HE858643
	Trehalose-6-phosphate synthase	<i>Aedes aegypti</i> / XP_001657813.1	1.00E-22	cyt	HE858687
	Trehalose-6-phosphate synthase 1	<i>Culex quinquefasciatus</i> / XP_001850996.1	2.00E-06	cyt	HE858920
	2,5-diketo-D-gluconic acid reductase A	<i>Rhodococcus erythropolis</i> SK121 / ZP_04383522.1	2.00E-05	cyt	HE859383
	3,4-dihydroxy-2-butanone-4-phosphate synthase	<i>Haemophilus influenzae</i> HK1212 / ZP_06222660.1	5.00E-16	cyt	HE858684
	3,4-dihydroxy-2-butanone-4-phosphate synthase	<i>Haemophilus influenzae</i> HK1212 / ZP_06222660.1	1.00E-13	cyt	HE858659
	3,4-dihydroxy-2-butanone-4-phosphate synthase	<i>Haemophilus influenzae</i> HK1212 / ZP_06222660.1	3.00E-20	cyt	HE858708
	Amylo-1, 6-glucosidase, 4-alpha-glucanotransferase	<i>Danio rerio</i> / NP_001166124.1	2.00E-39	cyt	HE858838
	Arginine kinase	<i>Philaethria wernickei</i> / ACZ26817.1	1.00E-42	cyt	HE858779
	Arginine kinase	<i>Glossina morsitans morsitans</i> / ADD19663.1	6.00E-67	cyt	HE859110
	Putative enolase	<i>Lutzomyia longipalpis</i> / ABV60328.1	3.00E-39	mit	HE858686
	Putative enolase	<i>Myrmecocystus depilis</i> / ABY55706.1	5.00E-08	mit	HE859204

Continuation

Functional category	Putative Identity	Species and accession number	E value	D*	Pyropia accession number
4. Antioxidant, chaperone and defense factors	Glutamate dehydrogenase	<i>Bombyx mori</i> / NP_001040245.1	3.00E-43	mit	HE859167
	Glycerol kinase	<i>Candidatus Liberibacter americanus</i> str. Sao Paulo / ACD87749.1	4.00E-22	mit	HE858676
	Short-chain dehydrogenase	<i>Aedes aegypti</i> / XP_001663676.1	1.00E-17	cyt	HE859135
	Peroxiredoxin (PRX) family, typical 2-Cys	<i>Pyropia yezoensis</i> / YP_536954.1	3.00E-20	chl	HE858809
	Arachidonate 5-lipoxygenase	<i>Strongylocentrotus purpuratus</i> / XP_001198535.1	1.00E-13	cyt	HE858870
	Glutathione S-transferase sigma 5	<i>Locusta migratoria</i> / AEB91977.1	7.00E-41	n.d.	HE858928
	28 kDa heat- and acid-stable phosphoprotein	<i>Harpegnathos saltator</i> / EFN84845.1	2.00E-13	cyt	HE858971
	Apolipoprotein D	<i>Culex quinquefasciatus</i> / XP_001848365.1	2.00E-43	n.d.	HE858692
	Probable Bax Inhibitor 1	<i>Harpegnathos saltator</i> / EFN76935.1	3.00E-14	n.d.	HE859251
	Ferritin heavy chain-like	<i>Ailuropoda melanoleuca</i> / XP_002929594.1	1.00E-06	cyt	HE859190
	Ferritin heavy chain-like protein	<i>Phlebotomus papatasi</i> / ABV44737.1	1.00E-33	cyt	HE858813
	Small heat shock protein IbpA	<i>Trichinella pseudospiralis</i> / ABJ55915.1	2.00E-28	mit	HE858802
	Heat shock protein 23 beta	<i>Ceratitidis capitata</i> / ACG58884.1	1.00E-05	cyt	HE858848
	Heat shock protein 27	<i>Drosophila melanogaster</i> / NP_524000.1	7.00E-05	cyt	HE859216
	Heat shock protein 27	<i>Drosophila bipectinata</i> / AEB40325.1	5.00E-15	cyt	HE858649
	Heat shock protein 27	<i>Drosophila parabiepectinata</i> / AEB40323.1	5.00E-08	cyt	HE858750
	Heat shock protein 27	<i>Ceratitidis capitata</i> / ACD76913.1	2.00E-29	cyt	HE859304
	Heat shock protein 40	<i>Bactrocera dorsalis</i> / ADO30472.1	6.00E-17	n.d.	HE858885
	Heat shock protein 70	<i>Porphyra purpurea</i> / NP_053925.1	5.00E-71	chl	HE858621
	Heat shock protein 70	<i>Pyropia haitanensis</i> / Q06W39.1	5.00E-26	chl	HE858874
	Heat shock protein 70	<i>Pyropia haitanensis</i> / ACF71814.1	5.00E-36	cyt	HE858890
	Heat shock protein 70	<i>Ostreococcus lucimarinus</i> / XP_001417572.1	6.00E-65	n.d.	HE858770
	Heat shock protein 70	<i>Raphidiopsis brookii</i> D9 / ZP_06305357.1	9.00E-10	cyt	HE859296
	Heat shock protein 70B	<i>Dunaliella salina</i> / ACJ24805.1	3.00E-12	cyt	HE859318
	Chloroplast Heat shock protein 70	<i>Pyropia yezoensis</i> / ABF54971.1	2.00E-47	chl	HE858982
	Heat shock protein 70	<i>Gracilaria tenuistipitata</i> var. liui / YP_063608.1	4.00E-64	chl	HE858640
	Heat shock protein 70- type chaperone (chloroplast)	<i>Guillardia theta</i> / AAC35702.1	7.00E-54	chl	HE859033
	Heat shock protein 70C	<i>Ascaris suum</i> / ADY47984.1	3.00E-36	cyt	HE858821
	Heat shock protein 82	<i>Philodina roseola</i> / ACC43981.1	8.00E-20	n.d.	HE859067
	Heat shock protein 90	<i>Spodoptera exigua</i> / ACQ78181.1	9.00E-84	cyt	HE859242
	Heat shock protein 90	<i>Thitarodes pui</i> / ADA61011.1	2.00E-43	n.d.	HE858745
	Stress-induced-phosphoprotein 1	<i>Ciona intestinalis</i> / XP_002128875.1	3.00E-28	cyt	HE858818
5. Protein synthesis, processing and degradation	Cytochrome P450 4C1-like	<i>Acyrtosiphon pisum</i> / XP_001945361.2	8.00E-19	mit	HE859003
	Cytochrome P450 like_TBP	<i>Citrullus lanatus</i> / BAD26579.1	1.00E-12	mit	HE858931
	Putative cytochrome P450 like protein precursor	<i>Phillyrea latifolia</i> / CAK18871.1	8.00E-19	mit	HE858629
	20S proteasome alpha subunit	<i>Scylla paramamosain</i> / ACY66486.1	4.00E-34	cyt	HE859122
	26S protease regulatory subunit 8	<i>Acromyrmex echinator</i> / EGI70168.1	7.00E-57	cyt	HE858682
	26S proteasome non-ATPase regulatory subunit	<i>Aedes aegypti</i> / XP_001662445.1	2.00E-28	cyt	HE859333

Continuation

Functional category	Putative Identity	Species and accession number	E value	D*	Pyropia accession number
	26S proteasome non-ATPase regulatory subunit 14	<i>Aedes albopictus</i> / ADB43603.1	2.00E-47	cyt	HE858896
	26S proteasome regulatory complex subunit RPN5/PSMD12	<i>Glossina morsitans morsitans</i> / ADD19088.1	5.00E-28	cyt	HE859277
	Proteasome subunit beta type 3	<i>Culex quinquefasciatus</i> / XP_001843139.1	1.00E-33	cyt	HE859180
	Proteasome subunit alpha type 3	<i>Oncorhynchus mykiss</i> / ACO07671.1	2.00E-19	cyt	HE859121
	Eukaryotic translation initiation factor 3	<i>Culex quinquefasciatus</i> / XP_001851152.1	3.00E-37	cyt	HE859058
	Eukaryotic translation initiation factor 3 subunit 10	<i>Culex quinquefasciatus</i> / XP_001844367.1	6.00E-23	cyt	HE859324
	Putative 23S ribosomal RNA	<i>Vigna unguiculata</i> / CAO02532.1	4.93E-09	cyt	HE858807
	Putative ribosomal protein S3	<i>Vigna unguiculata</i> / CAO02550.1	8.00E-09	cyt	HE858624
	Ribosomal protein S8	<i>Polaribacter irgensii</i> 23-P / ZP_01119260.1	2.00E-28	cyt	HE858921
	30S ribosomal protein S10	<i>Polaribacter</i> sp.MED152 / ZP_05108264.1	5.00E-06	cyt	HE859208
	Ribosomal protein RPS3a	<i>Eurythoe complanata</i> / ABW23199.1	2.00E-68	mit	HE858801
	40S ribosomal protein S4	<i>Mytilus edulis</i> / ABA55738.1	9.00E-47	cyt	HE859005
	Ribosomal protein S9	<i>Haliotis discus discus</i> / ABX26127.1	5.00E-62	mit	HE858760
	Ribosomal protein S13	<i>Xenopus (Silurana) tropicalis</i> / NP_001016602.1	1.00E-62	cyt	HE859014
	Ribosomal protein S15 isoform A	<i>Lysiphlebus testaceipes</i> / AAX62477.1	7.00E-56	cyt	HE858833
	40S ribosomal protein S16	<i>Glossina morsitans morsitans</i> / ADD20564.1	6.00E-34	cyt	HE859173
	Putative 40S ribosomal protein RPS16	<i>Novocrania anomala</i> / ACD65104.1	2.00E-67	cyt	HE858899
	40S ribosomal protein S30	<i>Aedes aegypti</i> / XP_001653913.1	2.00E-14	cyt	HE858808
	40S ribosomal protein SA-like	<i>Nasonia vitripennis</i> / XP_001608082.1	7.00E-56	nuc	HE859192
	Ribosomal protein RPL14	<i>Arenicola marina</i> / ABW23170.1	6.00E-55	mit	HE859118
	Ribosomal protein L32	<i>Lepidochitona cinerea</i> / ACR24953.1	8.00E-48	cyt	HE859166
	60S ribosomal protein L6	<i>Harpegnathos saltator</i> / EFN79475.1	7.00E-26	cyt	HE858776
	60S ribosomal protein L10-3	<i>Zea mays</i> / NP_001149336.1	1.00E-11	cyt	HE858638
	60S ribosomal protein L11	<i>Glossina morsitans morsitans</i> / ADD20563.1	4.00E-35	mit	HE858806
	60S ribosomal protein L12	<i>Zea mays</i> / ACG35173.1	3.00E-38	nuc	HE859020
	Ribosomal protein RPL17	<i>Eurythoe complanata</i> / ABW23221.1	3.00E-16	nuc	HE858940
	Large subunit ribosomal protein 23	<i>Pristionchus pacificus</i> / ABR87202.1	2.00E-51	cyt	HE859259
	Putative 60S ribosomal protein RPL27	<i>Novocrania anomala</i> / ACD65128.1	2.00E-39	mit	HE858729
	Ribosomal protein L10a, component of cytosolic 80S ribosome and 60S large subunit	<i>Chlamydomonas reinhardtii</i> / XP_001699807.1	2.00E-46	cyt	HE858749
	Putative 23S ribosomal RNA	<i>Vigna unguiculata</i> / CAO02532.1	3.00E-13	mit	HE858674
	Putative ribosomal protein S3	<i>Vigna unguiculata</i> / CAO02550.1	4.00E-13	mit	HE859345
	Putative ribosomal protein S3	<i>Vigna unguiculata</i> / CAO02550.1	8.00E-15	mit	HE859397
	Ubiquitin C	<i>Schistosoma japonicum</i> / CAX79699.1	2.00E-53	cyt	HE858679
	Ubiquitin C	<i>Equus caballus</i> / NP_001075331.1	5.00E-24	cyt	HE858977
	Putative ubiquitin C variant 1	<i>Taeniopygia guttata</i> / ACH45550.1	7.00E-38	cyt	HE858934
	Predicted protein (ubiquitin isoform 1)	<i>Physcomitrella patens</i> subsp. <i>patens</i> / XP_001764949.1	4.00E-14	chl	HE858820
	Ubiquitin-conjugating enzyme E2	<i>Zea mays</i> / NP_001140410.1	3.00E-33	chl	HE858941
	E3 Ubiquitin-protein ligase UHRF1-Like	<i>Meleagris gallopavo</i> / XP_003213379.1	2.00E-08	cyt	HE858646

Continuation

Functional category	Putative Identity	Species and accession number	E value	D*	Pyropia accession number
	Hypothetical protein 382	<i>Pyropia yezoensis</i> / YP_537017.1	1.00E-14	cyt	HE858985
	Molybdopterin biosynthesis protein	<i>Porphyra purpurea</i> / NP_053945.1	9.00E-10	cyt	HE859052
	Peptidase membrane zinc metallopeptidase	<i>Pelodictyon phaeoclathratiforme</i> BU-1 / YP_002018463.1	3.00E-12	cyt	HE858858
	Protein disulfide isomerase	<i>Dictyostelium discoideum</i> AX4 / XP_635206.1	2.00E-16	cyt	HE858804
	Serine peptidase 2	<i>Radix peregra</i> / ABL67951.1	1.00E-22	ext	HE858888
	Serine protease	<i>Aedes aegypti</i> / XP_001655711.1	1.00E-07	cyt	HE859141
	Serine-type endopeptidase	<i>Culex quinquefasciatus</i> / XP_001846627.1	8.00E-12	cyt	HE858654
	Putative serine-type endopeptidase	<i>Aedes aegypti</i> / XP_001663439.1	4.00E-18	cyt	HE858699
	Signal peptidase complex catalytic subunit SEC11A	<i>Danio rerio</i> / NP_001002521.1	7.00E-08	cyt	HE859034
	Signal peptidase complex subunit 3	<i>Bombyx mori</i> / NP_001091763.1	3.00E-32	cyt	HE859075
	Signal recognition particle subunit Srp68	<i>Glossina morsitans morsitans</i> / ADD18348.1	4.00E-10	cyt	HE858914
	Signal sequence receptor, beta-like	<i>Saccoglossus kowalevskii</i> / XP_002740523.1	6.00E-14	cyt	HE858810
	T-complex protein 1 subunit epsilon	<i>Harpegnathos saltator</i> / EFN84860.1	2.00E-54	cyt	HE859168
	Translation initiation factor	<i>Anopheles gambiae</i> str. PEST / XP_316499.2	2.00E-30	cyt	HE859078
	Protein translation factor	<i>Griffithsia japonica</i> / AAM93956.1	8.00E-40	cyt	HE858711
	Translation initiation factor 2 gamma subunit	<i>Allacma fusca</i> / CAG29667.1	1.00E-24	cyt	HE859111
	Histidine-tRNA synthetase	<i>Porphyra purpurea</i> / NP_053958.1	1.00E-47	chl	HE858867
	Histidyl-tRNA synthetase	<i>Moorea producens</i> 3L / ZP_08428961.1	2.00E-10	chl	HE859222
	60S acidic ribosomal protein P0	<i>Haliotis diversicolor</i> / ABY87386.1	1.00E-58	cyt	HE859006
	60S acidic ribosomal protein P2	<i>Spodoptera frugiperda</i> / AAL62467.1	5.00E-18	cyt	HE859081
	Transcript antisense to ribosomal RNA protein 1 (Tar1p)	<i>Saccharomyces cerevisiae</i> S288c / NP_690845.1	2.00E-07	mit	HE859051
	Venom serine carboxypeptidase precursor	<i>Apis mellifera</i> / NP_001152775.1	1.00E-25	cyt	HE858876
	Cathepsin L1-like isoform 1 (cysteine protease)	<i>Danio rerio</i> / XP_001341714.2	5.00E-07	mit	HE858698
	Cathepsin L2 cysteine protease	<i>Pinctada fucata</i> / ADC52431.1	8.00E-32	mit	HE859305
	Cysteine proteinase cathepsin F	<i>Glossina morsitans morsitans</i> / ADD19167.1	3.00E-22	cyt	HE859086
	Elongation factor 1 gamma	<i>Caenorhabditis brenneri</i> / ACE00305.1	1.00E-13	cyt	HE859382
	Putative elongation factor 1 gamma	<i>Ixodes scapularis</i> / XP_002410199.1	5.00E-39	cyt	HE859183
	Elongation factor 1-beta	<i>Culex tarsalis</i> / ACJ64291.1	5.00E-26	cyt	HE858793
	Elongation factor 1 beta	<i>Simulium nigritanum</i> / ACZ28393.1	2.00E-30	cyt	HE858987
	Elongation factor 1-beta	<i>Artemia salina</i> / P12262.3	4.00E-16	cyt	HE858954
	Elongation factor-1 gamma	<i>Trichinella spiralis</i> / XP_003375107.1	2.00E-09	cyt	HE859353
	Elongation factor-1 gamma	<i>Bothriocyrtum californicum</i> / ABG88914.1	6.00E-30	cyt	HE859385
	Elongation factor-1 gamma	<i>Aliatypus plutonis</i> / ABG88916.1	5.00E-44	cyt	HE859214
	Elongation factor-1 gamma	<i>Deinopis spinosa</i> / ABG88956.1	3.00E-12	cyt	HE859387
	Elongation factor-1 gamma	<i>Aptostichus</i> sp. 4 NAA-2006 / ABG88917.1	2.00E-64	cyt	HE859398
	Elongation factor-2	<i>Harbansus paucichelatus</i> / AAR01294.1	5.00E-12	cyt	HE859262
	Peptidyl prolyl cis-trans isomerase B	<i>Conus novaeollandiae</i> / ADC80506.1	2.00E-61	cyt	HE858895
	Peptidyl-prolyl cis-trans isomerase	<i>Culex quinquefasciatus</i> / XP_001846632.1	2.00E-23	cyt	HE859125
	Peptidyl-prolyl isomerase-1	<i>Gryllus firmus</i> / ACD69575.1	6.00E-12	cyt	HE859254

Continuation

Functional category	Putative Identity	Species and accession number	E value	D*	Pyropia accession number
6. Cell motility	Phenylalanyl-tRNA synthetase beta chain	<i>Porphyra purpurea</i> / NP_053956.1	9.00E-42	chl	HE859109
	Ribosome biogenesis protein Nsa2 homolog	<i>Ictalurus punctatus</i> / NP_001187984.1	3.00E-84	nuc	HE858869
	Metalloprotease inhibitor	<i>Medicago truncatula</i> / XP_003616487.1	3.00E-52	cyt	HE858630
	Intermediate filament protein	<i>Biomphalaria glabrata</i> / AAZ39528.1	2.00E-25	cyt	HE859009
	Myomesin	<i>Aedes aegypti</i> / XP_001655591.1	1.00E-13	cyt	HE859273
	Myosin light chain	<i>Gryllotalpa orientalis</i> / AAW22542.1	2.00E-43	cyt	HE858829
	Myosin 1 light chain	<i>Palaemonetes varians</i> / ACR54116.1	3.00E-16	cyt	HE859189
	Myosin heavy chain isoform 3	<i>Daphnia pulex</i> / EFX87106.1	8.00E-35	n.d.	HE859089
	Paramyosin, short form	<i>Harpegnathos saltator</i> / EFN75172.1	9.00E-68	n.d.	HE858678
	Paramyosin, short form	<i>Camponotus floridanus</i> / EFN63022.1	6.00E-35	n.d.	HE859063
	Tropomodulin	<i>Culex quinquefasciatus</i> / XP_001867000.1	1.00E-28	cyt	HE859137
	Tropomodulin, isoform K	<i>Drosophila melanogaster</i> / NP_001189319.1	2.00E-33	cyt	HE858787
	Tropomyosin-2 isoform 1	<i>Bombyx mori</i> / NP_001103782.1	1.00E-60	cyt	HE858718
	Actin	<i>Pyropia yezoensis</i> / BAB64309.1	1.00E-14	cyt	HE859049
	Actin	<i>Apis mellifera</i> / XP_003251465.1	6.00E-43	cyt	HE858761
	Actin-2, partial	<i>Ascaris suum</i> / ADY47269.1	9.00E-73	cyt	HE858791
	Actin 4	<i>Tetranychus urticae</i> / ACN53544.1	8.00E-35	cyt	HE859406
	Actin 57B	<i>Glossina morsitans morsitans</i> / ADD19431.1	7.00E-13	cyt	HE858814
	Actin 88F	<i>Drosophila melanogaster</i> / NP_524367.1	5.00E-78	cyt	HE858619
	Actin	<i>Timema tahoe</i> / ADX66580.1	1.00E-74	cyt	HE858642
	Actin E2	<i>Drosophila virilis</i> / AAK25829.1	2.00E-41	cyt	HE859379
	Alpha actinin, isoform A	<i>Drosophila melanogaster</i> / NP_477484.2	1.00E-29	cyt	HE859142
	Beta-actin	<i>Diablocatanops pinguis</i> / ACV32627.1	5.00E-78	cyt	HE858722
	Beta-actin	<i>Diablocatanops pinguis</i> / ACV32627.1	9.00E-28	cyt	HE858763
	Beta-actin	<i>Channa punctata</i> / AEA50896.1	9.00E-40	cyt	HE859079
	Actin 87E	<i>Glossina morsitans morsitans</i> / ADD19714.1	4.00E-76	cyt	HE858840
	Actin type 1	<i>Ostrea edulis</i> / CAL69229.1	1.00E-72	cyt	HE859022
	Actin, cytoplasmic 2-like isoform 1	<i>Callithrix jacchus</i> / XP_002758560.1	2.00E-54	cyt	HE858700
	Alpha-tubulin	<i>Boltenia villosa</i> / AAM76122.1	2.00E-45	cyt	HE858975
	Ankyrin-1-like	<i>Apis mellifera</i> / XP_397331.2	3.00E-60	cyt	HE859178
	Dynein heavy chain	<i>Culex quinquefasciatus</i> / XP_001843519.1	2.00E-04	cyt	HE859030
	Stretchin-Mlck, isoform L	<i>Drosophila melanogaster</i> / NP_001188951.1	3.00E-06	cyt	HE858663
	Up-regulated during skeletal muscle growth protein 5 – like	<i>Acyrtosiphon pisum</i> / XP_003247952.1	6.00E-04	cyt	HE858948
	Inhibitor of apoptosis protein	<i>Bombyx mori</i> / AAN46650.1	6.00E-20	cyt	HE858854
7. Cell growth and death	Translationally-controlled tumor protein	<i>Cyprinus carpio</i> / ABC59222.1	1.00E-11	cyt	HE858712
	Cathepsin D	<i>Pteropus pteropus</i> / AEI58895.1	3.00E-17	cyt	HE858983
	Death-associated small cytoplasmic leucine-rich protein	<i>Bombyx mori</i> / NP_001138799.1	3.00E-27	cyt	HE858839
	CHK1 checkpoint-like protein	<i>Perca flavescens</i> / ADX97237.1	1.00E-04	cyt	HE859132
8. Membrane transporters	Transmembrane channel-like 2	<i>Taeniopygia guttata</i> / XP_002196817.1	2.00E-04	n.d.	HE859194

Continuation

Functional category	Putative Identity	Species and accession number	E value	D*	Pyropia accession number
	Protein-export membrane protein	<i>Psychroflexus torquis</i> ATCC 700755 / ZP_01252230.1	4.00E-19	n.d.	HE859043
	Secd/Secf	<i>Lactobacillus rhamnosus</i> LMS2-1 / ZP_04439763.1	6.00E-06	n.d.	HE859400
	RND superfamily resistance-nodulation-cell division: proton (H ⁺) antiporter				
	Sugar transporter	<i>Haliscomenobacter hydrossis</i> DSM 1100 / YP_004445152.1	2.00E-08	n.d.	HE858852
	Transport protein	<i>Paenibacillus</i> sp. HGF5 / ZP_08281960.1	1.00E-03	n.d.	HE859386
9. Vesicular transport and metabolism	Putative Ras-related protein Rab-2A	<i>Aedes aegypti</i> / XP_001650005.1	4.00E-15	gol	HE859113
10. Cell wall metabolism	RtnI1, isoform C	<i>Drosophila melanogaster</i> / NP_787988.1	1.00E-36	n.d.	HE859153
	Transport protein Sec61 subunit alpha 2	<i>Culex quinquefasciatus</i> / XP_001850184.1	3.00E-113	n.d.	HE859332
	Heparanase precursor	<i>Rattus norvegicus</i> / NP_072127.1	4.00E-05	cyt	HE858732
	Cell wall-associated hydrolase	<i>Escherichia</i> sp. 3253FAA / ZP_04532936.1	3.00E-21	cyt	HE859007
	Cell wall-associated hydrolase	<i>Burkholderia multivorans</i> ATCC 17616 / YP_001949468.1	1.00E-29	cyt	HE858683
	Cell wall-associated hydrolase	<i>Microscilla marina</i> ATCC 23134 / ZP_01689674.1	7.00E-20	cyt	HE858990
	Cell wall-associated hydrolase	<i>Roseobacter</i> sp. AzwK-3b / ZP_01900972.1	5.00E-16	cyt	HE859054
	Cell wall-associated hydrolase	<i>Escherichia</i> sp.3253FAA / ZP_04532936.1	3.00E-12	cyt	HE859275
	Cell wall-associated hydrolase	<i>Helicobacter canadensis</i> / ZP_07804970.1	2.00E-05	cyt	HE859290
	Cell wall-associated hydrolase	<i>Streptomyces</i> sp. e14 / ZP_06708251.1	4.00E-09	cyt	HE859401
11. Photosynthesis and chloroplast proteins	Glycoside hydrolases	<i>Aedes aegypti</i> / XP_001650341.1	1.00E-58	cyt	HE858995
	Glycosyl transferase family protein	<i>Acidiphilium cryptum</i> JF-5 / YP_001233531.1	8.00E-06	cyt	HE858973
	30S ribosomal protein S13	<i>Pyropia yezoensis</i> / ABJ91310.1	2.00E-26	chl	HE859292
	Ribosomal protein L3	<i>Porphyra purpurea</i> / NP_053924.1	2.00E-25	chl	HE858764
	Ribosomal protein L13	<i>Porphyra purpurea</i> / NP_053902.1	3.00E-43	chl	HE858795
	Ribosomal protein L35	<i>Pyropia yezoensis</i> / YP_536952.1	7.00E-07	chl	HE859002
	Photosystem I P700 chlorophyll A apoprotein A1	<i>Arthrospira platensis</i> str. Paraca / ZP_06383599.1	4.00E-40	chl	HE859186
	Photosystem I P700 chlorophyll A apoprotein A1	<i>Bangia fuscopurpurea</i> / AAM62007.1	7.00E-70	chl	HE858916
	Photosystem I P700 chlorophyll A apoprotein A1	<i>Porphyra purpurea</i> / NP_053894.1	1.00E-52	chl	HE859114
	Photosystem II cytochrome C550	<i>Porphyra purpurea</i> / NP_053809.1	1.00E-37	chl	HE858727
	Photosystem II D2 protein	<i>Pyropia yezoensis</i> / YP_537038.1	1.00E-06	chl	HE859365
	Photosystem II protein D1	<i>Pyropia yezoensis</i> / YP_536893.1	9.00E-103	chl	HE858641
	Photosystem II protein D1	<i>Porphyra purpurea</i> / NP_053822.1	1.00E-93	chl	HE858733
	Photochlorophyllide reductase subunit B	<i>Porphyra purpurea</i> / NP_053888.1	9.00E-35	chl	HE858824
	Photochlorophyllide reductase subunit L	<i>Porphyra purpurea</i> / NP_053797.1	2.00E-32	chl	HE859157
	Photochlorophyllide reductase subunit N	<i>Porphyra purpurea</i> / NP_053798.1	2.00E-27	chl	HE859287
	Phycobilisome linker polypeptide	<i>Cyanothece</i> sp. PCC 7822 / YP_003889235.1	1.00E-03	chl	HE859143
	Phycocerythrin beta subunit	<i>Porphyra purpurea</i> / NP_053977.1	3.00E-08	chl	HE858627
	Phycocerythrin beta subunit	<i>Ceramium boydenii</i> / AAM88398.1	1.00E-50	chl	HE858639
	Putative rubisco expression protein	<i>Rhodomonas salina</i> / YP_001293520.1	3.00E-75	chl	HE859066
	Allophycocyanin subunit alpha	<i>Microcystis aeruginosa</i> NIES-843 / YP_001656041.1	1.00E-12	chl	HE858886
	ATP synthase CF1 delta subunit	<i>Porphyra purpurea</i> / NP_053853.1	5.00E-12	chl	HE858967
	ATP synthase CF0 A subunit	<i>Pyropia yezoensis</i> / YP_536928.1	9.00E-11	chl	HE859243

Continuation

Functional category	Putative Identity	Species and accession number	E value	D*	Pyropia accession number
12. Respiration and mitochondrial proteins	ATP synthase CF0 subunit I	<i>Vigna unguiculata</i> / CAO02552.1	7.00E-09	chl	HE858944
	Beta-Ig-H3/fasciclin	<i>Acidiphilium cryptum</i> JF-5 / YP_001235357.1	3.00E-07	chl	HE858860
	Clp protease ATP binding subunit	<i>Pyropia yezoensis</i> / YP_537014.1	2.00E-56	chl	HE858999
	Cytochrome P450 like_TBP	<i>Nicotiana tabacum</i> / BAA10929.1	5.00E-30	chl	HE858796
	Glycine cleavage system protein H	<i>Hydrogenivirga</i> sp. 128-5-R1-1 / ZP_02176878.1	3.00E-05	chl	HE859272
	Mitochondrial processing peptidase beta subunit	<i>Glossina morsitans morsitans</i> / ADD20239.1	4.00E-62	mit	HE858961
	Cytochrome B-C1 complex subunit 9-like	<i>Anolis carolinensis</i> / XP_003230444.1	1.00E-05	mit	HE858900
	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8 mitochondrial	<i>Nasonia vitripennis</i> / NP_001161450.1	4.00E-14	mit	HE858968
	NADH dehydrogenase subunit 1	<i>Littorina saxatilis</i> / CAM57998.1	6.00E-52	mit	HE858930
	NADH dehydrogenase subunit 2	<i>Fusiturris similis</i> / YP_003204746.1	8.00E-12	mit	HE858904
	NADH dehydrogenase subunit 2	<i>Porphyra purpurea</i> / NP_049304.1	5.00E-04	mit	HE859276
	NADH-ubiquinone oxidoreductase 39 kDa subunit	<i>Culex quinquefasciatus</i> / XP_001865472.1	2.00E-37	mit	HE858694
	NADH-ubiquinone oxidoreductase Fe-S protein 2 (Ndufs2)	<i>Aedes aegypti</i> / XP_001655316.1	2.00E-69	mit	HE859008
	Cytochrome oxidase subunit I	<i>Lucilia cuprina</i> / CBX78584.1	2.00E-52	mit	HE858780
	Cytochrome oxidase subunit I	<i>Dicranomyia variabilis</i> / ABV71159.1	1.00E-35	mit	HE858720
	Cytochrome oxidase subunit I	<i>Littorina saxatilis</i> / CAA10593.1	1.00E-65	mit	HE858856
	Cytochrome oxidase subunit I	<i>Echinolittorina novaezelandiae</i> / CAF22173.1	1.00E-61	mit	HE859031
	Cytochrome oxidase subunit II	<i>Geranomyia advena</i> / ABV71230.1	5.00E-42	mit	HE858740
	Cytochrome oxidase subunit II	<i>Ceratitidis ditissima</i> / ADB55739.1	3.00E-10	mit	HE859328
	Cytochrome oxidase subunit II	<i>Dicranomyia stygipennis</i> / ABV71214.1	8.00E-06	mit	HE858917
	Cytochrome oxidase subunit II	<i>Dicranomyia swezeyi</i> / ABV71215.1	2.00E-10	mit	HE859117
	Cytochrome oxidase subunit II	<i>Littorina saxatilis</i> / CAA10594.1	2.00E-22	mit	HE858884
	Cytochrome C oxidase subunit 3	<i>Potamopyrgus antipodarum</i> / ADB93443.1	1.00E-65	mit	HE858669
	Cytochrome C oxidase subunit III	<i>Cymatium parthenopeum</i> / YP_003204809.1	7.00E-25	mit	HE859331
	Cytochrome C oxidase subunit III	<i>Aedes albopictus</i> / YP_194916.1	2.00E-38	mit	HE858725
	Cytochrome C oxidase subunit IV	<i>Drosophila simulans</i> / ABM88269.1	5.00E-29	mit	HE858714
	Cytochrome C oxidase subunit Va/COX6	<i>Simulium nigritanum</i> / ACZ28403.1	1.00E-32	mit	HE858693
	Cytochrome C oxidase polypeptide VIa	<i>Oncorhynchus mykiss</i> / ACO08369.1	4.00E-08	mit	HE858746
	Cytochrome C oxidase subunit 6a polypeptide 1	<i>Saccoglossus kowalevskii</i> / XP_002738468.1	1.00E-22	mit	HE859201
	F1F0-ATP synthase subunit C/ATP9/proteolipid	<i>Simulium nigritanum</i> / ACZ28249.1	1.00E-14	mit	HE858739
	F1F0-ATP synthase subunit epsilon/ATP15	<i>Glossina morsitans morsitans</i> / ADD20505.1	4.00E-16	mit	HE858689
	F1F0-ATP synthase subunit OSCP/ATP5	<i>Glossina morsitans morsitans</i> / ADD18988.1	3.00E-54	mit	HE858984
	Mitochondrial matrix protein P32	<i>Bombyx mori</i> / ABD36320.1	2.00E-11	mit	HE858994
	Mitochondrial succinate dehydrogenase cytochrome B subunit	<i>Culex quinquefasciatus</i> / XP_001859594.1	4.00E-10	mit	HE859236
	ADP / ATP carrier protein	<i>Ricinus communis</i> / XP_002517886.1	5.00E-41	mit	HE858960
	ADP / ATP translocase	<i>Apis mellifera</i> / NP_001010975.1	1.00E-58	mit	HE858665
	ATP synthase alpha subunit precursor	<i>Drosophila silvestris</i> / ABY55748.1	4.00E-36	mit	HE859342
	ATP synthase alpha subunit precursor	<i>Strongylocentrotus purpuratus</i> / NP_999743.1	4.00E-30	mit	HE858957

Continuation

Functional category	Putative Identity	Species and accession number	E value	D*	Pyropia accession number
13. Others	ATP synthase b	<i>Spodoptera exigua</i> / ACL77780.1	1.00E-10	mit	HE859072
	ATPSyn-beta	<i>Drosophila simulans</i> / XP_002105781.1	2.00E-36	mit	HE858786
	Mitochondrial ATP synthase delta subunit	<i>Aedes aegypti</i> / XP_001655448.1	1.00E-20	mit	HE859029
	ATP synthase delta subunit, isoform A	<i>Drosophila melanogaster</i> / NP_524402.1	2.00E-25	mit	HE858979
	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, O subunit	<i>Xenopus (Silurana) tropicalis</i> / NP_001037877.1	4.00E-17	mit	HE859056
	Cytochrome B	<i>Bolus brandaris</i> / YP_003204844.1	2.00E-38	mit	HE858728
	Cytochrome B	<i>Drosophila mojavensis</i> / DAA06245.1	5.00E-44	mit	HE858782
	Cytochrome B	<i>Liriomyza trifolii</i> / YP_003734909.1	3.00E-06	mit	HE859193
	Cytochrome C	<i>Glossina morsitans morsitans</i> / ADD19108.1	4.00E-34	mit	HE858933
	Cytochrome C1	<i>Tribolium castaneum</i> / EFA01361.1	3.00E-74	mit	HE858766
	Cytochrome C1	<i>Glossina morsitans morsitans</i> / ADD19528.1	1.00E-18	mit	HE859010
	ATP synthase F0 subunit 6	<i>Drosophila littoralis</i> / YP_002327406.1	3.00E-07	mit	HE858843
	H ⁺ transporting ATP synthase subunit e	<i>Bombyx mori</i> / NP_001091812.1	2.00E-08	mit	HE859061
	Predicted ubiquinol-cytochrome C reductase core protein I	<i>Oryctolagus cuniculus</i> / XP_002713430.1	4.00E-20	mit	HE859147
	Voltage-dependent anion-selective channel	<i>Culex quinquefasciatus</i> / XP_001842637.1	3.00E-47	mit	HE859045
	Putative senescence-associated protein	<i>Lilium longiflorum</i> / ABO20851.1	2.00E-15	n.d.	HE858777
	Putative senescence-associated protein	<i>Cupressus sempervirens</i> / ACA30301.1	1.00E-23	mit	HE859069
	Putative senescence-associated protein	<i>Perkinsus marinus</i> ATCC 50983/ XP_002771156.1	3.00E-07	n.d.	HE859266
	Senescence-associated protein	<i>Chlorella variabilis</i> / EFN58441.1	1.00E-05	mit	HE859270
	Kazal domain-containing peptide	<i>Anopheles darlingi</i> / ACI30205.1	1.00E-06	n.d.	HE859175
	Putative secreted protein 10 kDa	<i>Argas monolakensis</i> / ABI52743.1	7.00E-15	cyt	HE859146
	AGAP009754-PA (angiotensin-converting enzyme 4)	<i>Anopheles gambiae</i> / XP_001238056.2	9.00E-71	cyt	HE859209
	Another B-box affiliate, isoform A	<i>Drosophila melanogaster</i> / NP_611390.2	8.00E-26	ext	HE859269
	AGAP008459-PA (cuticular protein 9, low complexity family)	<i>Anopheles gambiae</i> / XP_001688982.1	1.00E-05	ext	HE858653
	AGAP009872-PA (cuticular protein 133, RR-1 family)	<i>Anopheles gambiae</i> str. PEST/ XP_001689165.1	1.00E-21	ext	HE858951
	Putative pupal cuticle protein	<i>Aedes aegypti</i> / XP_001661677.1	2.00E-17	ext	HE858625
	Fibroleukin-like	<i>Meleagris gallopavo</i> / XP_003201937.1	2.00E-26	cyt	HE858705
	Cuticular protein analogous to peritrophins 3-A1	<i>Acyrtosiphon pisum</i> / NP_001156724.1	2.00E-29	ext	HE859090
	Vitellogenin	<i>Lethocerus deyrollei</i> / BAG12118.1	3.06E-31	mit	HE858626
	Vitellogenin 1b	<i>Culex tarsalis</i> / ADH04225.1	2.00E-05	n.d.	HE858633
	Vitellogenin 1a	<i>Culex tarsalis</i> / ADH04224.1	2.00E-13	cyt	HE859037
	Vitellogenin 2a	<i>Culex tarsalis</i> / ADH04226.1	4.00E-22	chl	HE858675
	Vitellogenin A1	<i>Culex quinquefasciatus</i> / XP_001843136.1	2.00E-07	cyt	HE858849
	Vitellogenin 2b	<i>Culex tarsalis</i> / ADH04227.1	5.00E-36	cyt	HE858632
	Vitellogenin B	<i>Ochlerotatus atropalpus</i> / AAV31927.1	1.00E-06	ext	HE858657
	Vitellogenin C	<i>Toxorhynchites amboinensis</i> / AAV31932.1	4.00E-09	cyt	HE859170
	Vitellogenin C	<i>Anopheles albimanus</i> / AAV31933.1	5.00E-47	mit	HE858622
	Vitellogenin precursor	<i>Tenebrio molitor</i> / AAU20328.2	1.00E-06	n.d.	HE859404

Continuation

Functional category	Putative Identity	Species and accession number	E value	D*	<i>Pyropia</i> accession number
	Vitellogenin C	<i>Aedes polynesiensis</i> / AAV31926.1	7.00E-32	cyt	HE858631
	CDGSH iron sulfur domain-containing protein 2-like protein	<i>Camponotus floridanus</i> / EFN65337.1	2.00E-33	mit	HE858716
	Opsin-1	<i>Culex quinquefasciatus</i> / XP_001845697.1	8.00E-06	n.d.	HE859197
	Rhodopsin	<i>Aedes aegypti</i> / XP_001657619.1	4.00E-46	n.d.	HE858950
	Lethal(2)essential for life protein, l2efl	<i>Aedes aegypti</i> / XP_001663499.1	2.00E-36	n.d.	HE858887
	Similar to fibropellin Ia	<i>Strongylocentrotus purpuratus</i> / XP_001199485.1	3.00E-06	cyt	HE858672
	Cob(1)tyrinic acid a,c-diamide adenosyltransferase	<i>Selenomonas noxia</i> ATCC43541 / ZP_06604478.1	2.00E-05	n.d.	HE858754

revealed that ESTs with higher representation are involved in protein synthesis, processing and degradation (ca. 15%, *e.g.*, Clp-protease), photosynthesis and chloroplast structure (ca. 14.4%; *e.g.*, ferredoxin NADP⁺ reductase), respiration and mitochondria (ca. 13.1%), proteins involved in cell wall metabolism (ca. 10.6%, cell-wall hydrolases, glycosyl transferases and chitin deacetylases) and antioxidant activity, chaperone and defense factors (7.5%; thioredoxin (TRX), catalase and HSPs) (Table 1). Finally, with an important number of sequences it was not possible to find clear functional similarity with known proteins (ca. 11%).

DISCUSSION

The hydration-desiccation cycle in *P. columbina* generates an unbalance in the intracellular redox potential (Contreras-Porcia *et al.*, 2011a), a situation that must be controlled by a coordinated cascade of responses that are induced differentially in each condition. Our results show that, during hydration, predominantly expressed genes were those involved in protein metabolism. Among them, we highlight several ribosomal proteins, translation initiation factors and elongation factors, proteases, the proteasome system and several ubiquitins (Table 1). Of particular relevance is the ubiquitin-proteasome system, present in all eukaryotes, which is activated in response to several abiotic stress factors and participates in tolerance mechanisms by removing unfolded proteins and proteins damaged during oxidative stress (Dreher & Callis, 2007; Pena *et al.*, 2007; Kurepa *et al.*, 2008). In this functional group three types of peptidylprolyl cis-trans-isomerases (PPIases) were identified and found to be similar to those from Arthropoda and Mollusca. These proteins catalyze the cis-trans isomerisation of prolines (C₅H₉NO₂), and have been described in the processes of cellular signalling (with a calmodulin-binding

domain), regulation of gene transcription, and acting as chaperones and folding catalysts. Specifically, in the gastropod *Conus novaehollandiae* these proteins facilitate the oxidative folding of several neurotoxic peptides (Safavi-Hemami *et al.*, 2010). In plants, these proteins are involved in flowering (Wang *et al.*, 2010) and in controlling cell proliferation, since PPIase expression increases in the presence of cytokinin (Vittorioso *et al.*, 1998). PPIases are induced by wounding, heat and salt stress (Vucich & Gasser, 1996; Kurek *et al.*, 1999), and have been directly involved in membrane protein folding (*i.e.*, chloroplast and mitochondria) (Breiman *et al.*, 1992). Thus, the quick re-establishment of the normal condition after desiccation in *P. columbina* could be in part explained by both: i) the re-folding of structural proteins with important functions such as those involved in transcriptional regulation, and ii) by the removal of oxidized proteins. In fact, levels of oxidised proteins measured during the hydration-desiccation cycle were consistently lower in *P. columbina* compared to those in sensitive species (Contreras-Porcia *et al.*, 2011a, Flores-Molina *et al.*, unpublished data).

Proteins that form part of the energy metabolism were more highly represented during hydration [*i.e.*, Cytochrome C Oxidase subunits, NADH Dehydrogenase subunits, NADH-Ubiquinone Oxidoreductase subunits, F1F0-ATP Synthase subunits and ADP/ATP carrier proteins (Table 1)] than during desiccation stress (Table 2). Theoretically, maintaining the tolerance mechanisms required to buffer the effects of a stress imposes energy costs (*e.g.*, Zagdańska, 1995). Acclimation to oxidative stress depends on a high availability of NADPH and ATP, since most of the intracellular metabolic reactions require energy. For example, the ubiquitin-mediated system for intracellular protein degradation is ATP-dependent in all organisms (Ciechanover *et al.*, 1984). Additionally, the structure of the chromosomes plays a critical role in transcriptional regulation where the chromatin

Table 2. Functional category, identity and potential cellular destination of proteins and accession number of identified ESTs in *Pyropia columbina* under natural desiccation (UD library). Data from the hydrated library (UH) is presented in Table 1. *E* value: the best (lowest) Expect value (*E* value) of all alignments from that database sequence, D*: Putative destination.

Functional category	Putative identity	Species and accession number	<i>E</i> value	D*	<i>Pyropia</i> accession number
1. Signal transduction	Calmodulin	<i>Pyropia yezoensis</i> / ABN41559	8.00E-11	cyt	HE859451
	Enkurin TRPC channel interacting protein	<i>Saccoglossus kowalevskii</i> / NP_001158494	2.00E-11	cyt	HE859515
	Guanine nucleotide binding protein 3	<i>Dictyostelium discoideum</i> AX4 / XP_637356.1	2.00E-38	cyt	HE859861
	Hexamerin 2 beta	<i>Aedes aegypti</i> / XP_001659531.1	4.00E-27	cyt	HE859661
	Serine/threonine protein phosphatase 1	<i>Malus domestica</i> / AAD56010.1	4.00E-45	cyt	HE859472
2. Transcription, splicing and replication	RNA-directed DNA polymerase (reverse transcriptase)	<i>Ktedonobacter racemifer</i> DSM 44963 / ZP_06971690.1	8.00E-13	nuc	HE859542
	Reverse transcriptase	<i>Cyanothece</i> sp. ATCC 51142 / YP_001801525.1	9.00E-11	nuc	HE859708
	RNA-directed DNA polymerase (reverse transcriptase)	<i>Arthrospira maxima</i> CS-328 / ZP_03273642	1.00E-24	nuc	HE859482
	DNA-directed RNA polymerase, beta subunit	<i>Crocospaera watsonii</i> WH 8501 / ZP_00516049.1	8.00E-48	nuc	HE859717
	RNA polymerase alpha subunit	<i>Porphyras purpurea</i> / NP_053903.1	3.00E-09	nuc	HE859795
	Argonaute2 (AGO2)	<i>Albugo laibachii</i> Nc14 / CCA27516.1	4.00E-21	cyt	HE859580
	Predicted metal-dependent RNase	<i>Streptococcus pyogenes</i> M49 591 / ZP_00366321.1	8.00E-17	nuc	HE859892
	Group II intron reverse transcriptase/maturase	<i>Streptococcus dysgalactiae</i> subsp. <i>dysgalactiae</i> ATCC 27957 / EFY02523.1	8.00E-11	nuc	HE859734
	Histone H3.3	<i>Harpegnathos saltator</i> / EFN83153.1	4.00E-38	nuc	HE859642
	Retrotransposon protein	<i>Oryza sativa</i> Indica Group / ABR26094.1	2.00E-22	nuc	HE859423
	Aconitate hydratase	<i>Camponotus floridanus</i> / EFN71522.1	2.00E-45	mit	HE859793
	Lipase	<i>Aedes aegypti</i> / XP_001652527.1	3.00E-25	ext	HE859755
	Fructose-1,6-biphosphatase F-II	<i>Griffithsia japonica</i> / AAP80707.1	2.00E-07	cyt	HE859780
3. Basal metabolism	Fructose-bisphosphate aldolase	<i>Sphingobacterium</i> sp. 21 / YP_004319198.1	6.00E-59	n.d.	HE859506
	Alpha-amylase B	<i>Culex quinquefasciatus</i> / XP_001846541.1	6.00E-26	ext	HE859517
	3-oxoacyl-ACP reductase	<i>Streptomyces avermitilis</i> MA-4680 / NP_824830.1	3.00E-06	ext	HE859599
	C-4 sterol methyl oxidase 2	<i>Nicotiana benthamiana</i> / AAQ83692.1	4.00E-11	mit	HE859431
	Amine oxidase	<i>Plasmodium yoelii yoelii</i> 17XNL / XP_730508.1	8.00E-13	cyt	HE859420
	Scavenger receptor cysteine-rich protein	<i>Culex quinquefasciatus</i> / XP_001866937.1	2.00E-05	n.d.	HE859437
	Vacuolar H ⁺ -ATPase C chain	<i>Pyropia tenera</i> / JC7151	4.00E-50	n.d.	HE859667
	Thioredoxin	<i>Hyperthermus butylicus</i> DSM 5456 / YP_001013307.1	2.00E-05	mit	HE859628
	Catalase	<i>Pyropia yezoensis</i> / ADO23652.1	4.00E-06	mit	HE859586
	Heme oxygenase	<i>Porphyras purpurea</i> / NP_053881.1	2.00E-05	chl	HE859574
4. Antioxidant, chaperone and defense factors	Cytochrome P450-Like TBP protein	<i>Lilium longiflorum</i> / ABO20848.1	4.00E-19	cyt	HE859591
	Heat shock protein 70	<i>Pyropia yezoensis</i> / YP_536996.1	2.00E-63	chl	HE859563
	dnaK gene product (Heat shock protein 70)	<i>Thermosynechococcus elongatus</i> BP-1 / NP_682523.1	1.00E-39	chl	HE859424
	Heat shock protein Hsp70	<i>Raphidiopsis brookii</i> D9 / ZP_06305357.1	2.00E-24	chl	HE859837
	Molecular chaperones HSP70/HSC70, HSP70	<i>Ectocarpus siliculosus</i> / CBN79394.1	2.00E-82	mit	HE859570
	Heat shock protein Hsp70	<i>Cylindrospermopsis raciborskii</i> CS-505 / ZP_06308882.1	3.00E-47	chl	HE859573
	Heat shock protein 90, partial	<i>Thraustotheca clavata</i> / AAX10950.1	6.00E-79	nuc	HE859869
	Heat shock protein 90	<i>Cellulophaga algicola</i> DSM 14237 / YP_004165199.1	1.00E-61	cyt	HE859588

Continuation

Functional category	Putative identity	Species and accession number	E value	D*	Pyropia accession number
5. Protein synthesis, processing and degradation	Molecular chaperones GRP78/Bip/KAR2, HSP70 superfamily	<i>Ectocarpus siliculosus</i> / CBJ48460.1	1.00E-34	cyt	HE859467
	Ubiquitin	<i>Oncorhynchus mykiss</i> / ACO07546.1	4.00E-04	mit	HE859794
	Ubiquitin protein	<i>Triticum aestivum</i> / AAQ08322	3.00E-36	cyt	HE859488
	Ubiquitin	<i>Phanerochaete chrysosporium</i> / CAA83244	1.00E-06	cyt	HE859630
	Ubiquitin	<i>Drosophila persimilis</i> / XP_002023314.1	8.00E-04	cyt	HE859858
	Ubiquitin-conjugating enzyme E2-17 kDa	<i>Zea mays</i> / ACG37110.1	7.00E-39	cyt	HE859634
	Proteasome subunit beta type-6	<i>Phytophthora infestans</i> T30-4 / XP_002901169.1	6.00E-11	cyt	HE859840
	Molybdopterin biosynthesis protein	<i>Porphyra purpurea</i> / NP_053945.1	2.00E-35	chl	HE859454
	30S ribosomal protein S18	<i>Gemella sanguinis</i> M325 / ZP_08260903	2.00E-10	n.d.	HE859511
	Ribosomal protein L35	<i>Pyropia yezoensis</i> / YP_536952.1	1.00E-06	chl	HE859735
	Protein translation factor	<i>Griffithsia japonica</i> / AAM93956	2.00E-47	cyt	HE859466
	Eukaryotic translation elongation factor 1 alpha 1	<i>Chlamydomonas reinhardtii</i> / XP_001696568.1	2.00E-58	cyt	HE859552
	EF2, translation elongation factor 2	<i>Ectocarpus siliculosus</i> / CBJ32863.1	1.00E-36	cyt	HE859801
	Elongation factor-like protein	<i>Pseudoperkinsus tapetis</i> / ADE62443.1	3.00E-35	cyt	HE859728
	Clp protease ATP binding subunit	<i>Pyropia yezoensis</i> / YP_537014.1	2.00E-58	cyt	HE859649
	Cysteine synthase	<i>Porphyra purpurea</i> / AAP97124.1	1.00E-74	cyt	HE859626
	Der F 3 allergen	<i>Dermatophagoides farinae</i> / AAP35076.1	2.00E-05	n.d.	HE859604
	trpB gene product	<i>Acaryochloris marina</i> MBIC11017 / YP_001519094.1	5.00E-10	n.d.	HE859936
	Isoleucyl-tRNA synthetase	<i>Lacinutrix</i> sp. 5H-3-7-4 / YP_004579101.1	2.00E-36	cyt	HE859750
	Prefolding-like protein	<i>Opisthacanthus cayaporum</i> / CAX51417.1	3.00E-22	cyt	HE859714
	Protease inhibitor G11A6	<i>Mayetiola destructor</i> / ABB70517.1	1.00E-05	cyt	HE859462
	Signal peptidase, catalytic subunit	<i>Chlamydomonas reinhardtii</i> / XP_001697614.1	1.00E-22	cyt	HE859469
6. Cell motility	Actin	<i>Helicoverpa armigera</i> / ADN84930.1	3.00E-24	cyt	HE859471
	Actin	<i>Pyropia yezoensis</i> / BAB64309.1	7.00E-13	cyt	HE859826
	Actin	<i>Undaria pinnatifida</i> / ADW66613.1	5.00E-97	cyt	HE859564
	Actin	<i>Pyropia yezoensis</i> / BAG71158.1	7.00E-24	cyt	HE859659
	Actin 1	<i>Paxillus involutus</i> / ABQ85639.1	9.00E-17	cyt	HE859928
	Beta-actin	<i>Mauremys mutica</i> / ADX86815.1	1.00E-21	cyt	HE859680
	Tropomyosin	<i>Crassostrea virginica</i> / AAC61869.1	3.00E-24	mit	HE859676
	Troponin T-1	<i>Drosophila melanogaster</i> / AAR24583.1	1.00E-05	cyt	HE859762
7. Cell growth and death	Beta-tubulin	<i>Phytophthora palmivora</i> / AAW58084	4.00E-64	cyt	HE859549
	Calponin	<i>Haliothis diversicolor</i> / ABU53030	9.00E-40	cyt	HE859480
	Cell division protein	<i>Pyropia yezoensis</i> / YP_537009.1	2.00E-17	chl	HE859522
	Cell division protein FtsA	<i>Lacinutrix</i> sp. 5H-3-7-4 / YP_004578825.1	6.00E-67	cyt	HE859665
	CHK1 checkpoint homolog	<i>Xenopus (Silurana) tropicalis</i> / CAJ83813.1	8.00E-04	cyt	HE859790
	CHK1 checkpoint-like protein	<i>Helicoverpa armigera</i> / ABK29471.1	1.00E-11	cyt	HE859593
	CHK1 checkpoint-like Protein	<i>Perca flavescens</i> / ADX97237.1	2.00E-10	cyt	HE859702
	ABC Transporter B family member 13	<i>Arabidopsis thaliana</i> / NP_174115	9.00E-16	n.d.	HE859505
8. Membrane transporters	ABC Transporter	<i>Thalassiosira pseudonana</i> CCMP1335 / XP_002288593	1.00E-06	n.d.	HE859535
	ATP-binding cassette (ABC) superfamily	<i>Ectocarpus siliculosus</i> / CBJ32723.1	1.00E-05	cyt	HE859694
	Endoplasmic reticulum vesicle transporter protein	<i>Arabidopsis thaliana</i> / NP_564162	6.00E-06	cyt	HE859464
9. Vesicular transport and metabolism					

Continuation

Functional category	Putative identity	Species and accession number	E value	D*	<i>Pyropia</i> accession number
10. Cell wall metabolism	GTPase SAR1	<i>Triticum aestivum</i> / ACD03831.1	2.00E-04	mit	HE859693
	Small GTP-binding protein	<i>Solanum lycopersicum</i> / AAA80679	8.00E-06	cyt	HE859512
	Cell wall-associated hydrolase	<i>Microscilla marina</i> ATCC 23134 / ZP_01689674.1	1.00E-17	cyt	HE859576
	Cell wall-associated hydrolase	<i>Escherichia</i> sp. 3_2_53FAA / ZP_04532936.1	3.00E-39	cyt	HE859695
	Cell wall-associated hydrolase	<i>Escherichia</i> sp. 3_2_53FAA / ZP_04532936.1	3.00E-09	cyt	HE859906
	Cell wall-associated hydrolase	<i>Escherichia</i> sp. 3_2_53FAA / ZP_04532936.1	2.00E-11	cyt	HE859921
	Cell wall-associated hydrolase	<i>Escherichia</i> sp. 3_2_53FAA / ZP_04532936.1	3.00E-13	cyt	HE859926
	Cell wall-associated hydrolase	<i>Escherichia</i> sp. 3_2_53FAA / ZP_04532936.1	2.00E-12	cyt	HE859929
	Cell wall-associated hydrolase	<i>Escherichia</i> sp. 3_2_53FAA / ZP_04532936.1	2.00E-15	cyt	HE859930
	Cell wall-associated hydrolase	<i>Microscilla marina</i> ATCC 23134 / ZP_01689674.1	5.00E-19	cyt	HE859413
	Putative cell wall-Associated hydrolase	<i>Campylobacter jejuni</i> subsp. <i>jejuni</i> BH-01-0142 / ZP_03217789.1	9.00E-11	cyt	HE859421
	Cell wall-associated hydrolase	<i>Prevotella bryantii</i> B14 / ZP_07059369.1	9.00E-25	cyt	HE859434
	Cell wall-associated hydrolase	<i>Burkholderia multivorans</i> ATCC17616 / YP_001949468.1	1.00E-25	cyt	HE859495
	Cell wall-associated hydrolase	<i>Erysipelothrix rhusiopathiae</i> ATCC 19414 / ZP_08082416.1	2.00E-18	cyt	HE859498
	Cell wall-associated Hydrolase	<i>Brucella abortus</i> NCTC 8038 / ZP_05822976.1	2.00E-14	mit	HE859566
	Cell wall-associated hydrolase	<i>Brucella suis</i> bv. 4 str. 40 / ZP_05839398.1	7.00E-19	cyt	HE859629
	Chitin deacetylase 7 precursor	<i>Tribolium castaneum</i> / NP_001104012.1	7.00E-10	cyt	HE859430
	Glycine cleavage system protein H	<i>Hydrogenivirga</i> sp. 128-5-R1-1 / ZP_02176878.1	7.00E-15	cyt	HE859890
	Glycosyl transferase	<i>Rhodospirillum photometricum</i> DSM 122 / CCG06616.1	1.00E-11	n.d.	HE859904
11. Photosynthesis and chloroplast proteins	Photosystem I P700 chlorophyll A apoprotein A1	<i>Porphyra purpurea</i> / NP_053894.1	8.00E-72	chl	HE859902
	Photosystem II protein D1	<i>Pyropia yezoensis</i> / YP_536893.1	1.00E-41	chl	HE859757
	Photosystem II protein D2	<i>Pyropia yezoensis</i> / YP_537038.1	4.00E-62	chl	HE859806
	Photosystem II protein D2	<i>Porphyra purpurea</i> / NP_053967	5.00E-93	chl	HE859500
	Ferredoxin NADP+ reductase	<i>Pisum sativum</i> / AAB59349.1	6.00E-05	chl	HE859729
	23S Ribosomal RNA	<i>Vigna unguiculata</i> / CAO02530.1	2.00E-09	chl	HE859509
	Ribosomal protein S3	<i>Vigna unguiculata</i> / CAO02550.1	5.00E-12	chl	HE859706
	Ribosomal protein L4	<i>Pyropia yezoensis</i> / YP_536993.1	5.00E-24	chl	HE859613
	30S ribosomal protein S13	<i>Trichodesmium erythraeum</i> IMS101 / YP_722618.1	4.00E-23	chl	HE859559
	Ribosomal protein L2	<i>Chlamydomonas reinhardtii</i> / ACS16390.1	1.00E-35	chl	HE859868
	30S ribosomal protein S12	<i>Porphyra purpurea</i> / NP_049316.1	6.00E-16	cyt	HE859584
	40S ribosomal protein S16	<i>Phytophthora infestans</i> T30-4 / XP_002899087.1	2.00E-13	cyt	HE859787
	50S ribosomal protein L3P	<i>Cyanobacterium</i> sp. UCYN-A / YP_003422083.1	3.00E-05	chl	HE859842
	50S ribosomal protein L3	<i>Prochlorococcus marinus</i> str. MIT 9515 / YP_001012054.1	5.00E-15	chl	HE859881
	50S ribosomal protein L4	<i>Lyngbya</i> sp. PCC 8106 / ZP_01619314.1	3.00E-12	chl	HE859818
	50S ribosomal protein L4	<i>Cyanothece</i> sp. PCC 7424 / YP_002378953.1	1.00E-06	chl	HE859828
	50S ribosomal protein L13	<i>Arthrospira platensis</i> NIES-39 / BAI90083.1	4.00E-37	chl	HE859578
	60S ribosomal protein L29-1	<i>Glycine max</i> / XP_003546330	2.00E-20	cyt	HE859481
	RNA polymerase beta subunit	<i>Porphyra purpurea</i> / NP_053860.1	3.00E-32	chl	HE859802

Continuation

Functional category	Putative identity	Species and accession number	E value	D*	Pyropia accession number
12. Respiration and mitochondrial proteins	ATP synthase beta subunit	<i>Trebouxia photobiont</i> / ABP98704.1	9.00E-11	chl	HE859691
	ATP synthase CF1 delta subunit	<i>Porphyra purpurea</i> / NP_053853.1	1.00E-14	chl	HE859731
	Cell division protein	<i>Porphyra purpurea</i> / NP_053937.1	5.00E-16	chl	HE859460
	Ferredoxin, chloroplast precursor	<i>Micromonas</i> sp. RCC299 / XP_002508604.1	2.00E-22	chl	HE859587
	Photochlorophyllide reductase subunit B	<i>Porphyra purpurea</i> / NP_053888.1	2.00E-12	chl	HE859810
	Photochlorophyllide reductase subunit B	<i>Pyropia yezoensis</i> / YP_536960.1	4.00E-52	chl	HE859555
	HESB-like domain-containing protein 2	<i>Zea mays</i> / NP_001149194	8.00E-38	chl	HE859536
	NADH dehydrogenase subunit 4L	<i>Porphyra purpurea</i> / NP_049322.1	7.00E-06	mit	HE859443
	NADH dehydrogenase subunit 7	<i>Coccomyxa subellipsoidea</i> C-169 / YP_004339024.1	3.00E-14	mit	HE859913
	Cytochrome C oxidase	<i>Ephedra viridis</i> / AAD01658.1	4.00E-09	mit	HE859770
	Cytochrome C oxidoreductase subunit II	<i>Porphyra purpurea</i> / NP_049294.1	7.00E-36	mit	HE859672
	Cytochrome C oxidase subunit III	<i>Tricola hortensis</i> / YP_003434166.1	2.00E-57	mit	HE859617
	Cytochrome C oxidase subunit IV	<i>Aedes aegypti</i> / XP_001663204.1	7.00E-16	mit	HE859683
	Ribosomal protein S3	<i>Porphyra purpurea</i> / NP_049318.1	4.00E-12	mit	HE859602
	Ribosomal protein S5	<i>Griffithsia japonica</i> / AAP80699.1	7.00E-64	mit	HE859456
	40S ribosomal protein S13-2	<i>Arabidopsis thaliana</i> / NP_567151.1	8.00E-43	mit	HE859742
	40S ribosomal protein S15	<i>Oryza sativa Japonica Group</i> / BAD30388	3.00E-24	mit	HE859501
	50S ribosomal protein L16	<i>Psychrobacter arcticus</i> 273-4 / YP_263793.1	5.00E-06	mit	HE859479
	50S ribosomal protein L16 conserved hypothetical protein	<i>Porphyra purpurea</i> / NP_049319.1	8.00E-15	mit	HE859664
		<i>Clostridium botulinum</i> NCTC 2916 / ZP_02955128.1	3.00E-08	mit	HE859899
	HNH endonuclease	<i>Arthrospira maxima</i> CS-328 / ZP_03274502.1	3.00E-15	mit	HE859427
	Reverse transcriptase	<i>Synechococcus</i> sp. PCC 7335 / ZP_05039776.1	4.00E-12	mit	HE859447
	Reverse transcriptase	<i>Arthrospira platensis</i> NIES-39 / BAI90930.1	3.00E-10	mit	HE859590
	Reverse transcriptase	<i>Porphyra purpurea</i> / NP_049296.1	7.00E-40	mit	HE859800
	F-Type H-ATPase beta subunit	<i>Ectocarpus siliculosus</i> / CBJ32298.1	1.00E-45	mit	HE859457
	Dehydration responsive protein	<i>Corchorus olitorius</i> / BAJ11784.1	1.00E-13	mit	HE859843
	Putative microvillar-like protein 5	<i>Phlebotomus perniciosus</i> / ADJ57676.1	6.00E-06	mit	HE859901
13. Others	Tumor differentially expressed protein	<i>Haploelma schmidtii</i> / ACH48223.1	2.00E-06	mit	HE859663
	Senescence-associated protein	<i>Cupressus sempervirens</i> / ACA30301.1	5.00E-33	cyt	HE859606
	Lipoprotein	<i>Lactobacillus rhamnosus</i> GG / CAR86203.1	5.00E-31	n.d.	HE859514
	Lipoprotein	<i>Lactobacillus rhamnosus</i> GG / CAR86203.1	9.00E-11	n.d.	HE859908
	Low-density lipoprotein receptor-related protein	<i>Ascaris suum</i> / ADY39765.1	2.00E-13	n.d.	HE859807
	Apolipoprotein D isoform 2	<i>Apis mellifera</i> / XP_623787.2	3.00E-08	n.d.	HE859474
	Putative apolipoprotein D	<i>Aedes aegypti</i> / XP_001660232.1	5.00E-10	n.d.	HE859648
	Dermatopontin 2	<i>Biomphalaria glabrata</i> / AAZ80785.1	1.00E-05	n.d.	HE859882
	Cysteine-rich venom protein	<i>Aedes aegypti</i> / XP_001655503.1	5.00E-07	n.d.	HE859698
	Venom allergen	<i>Aedes aegypti</i> / XP_001655382	1.00E-36	n.d.	HE859494
	Leucine rich protein	<i>Arachis hypogaea</i> / ABH09321.1	2.00E-12	n.d.	HE859468
	PgI protein	<i>Staphylococcus aureus</i> subsp. <i>aureus</i> MN8 / ZP_06947416	4.00E-24	n.d.	HE859493
	Short-chain collagen C4-like	<i>Amphimedon queenslandica</i> / XP_003391538.1	4.00E-22	cyt	HE859623
	Similar To DUF221 domain protein	<i>Leptosphaeria maculans</i> / CBX98851	1.00E-19	n.d.	HE859532
	Microvillar-like protein 5	<i>Phlebotomus perniciosus</i> / ADJ57676.1	4.00E-05	cyt	HE859418

Continuation

Functional category	Putative identity	Species and accession number	E value	D*	<i>Pyropia</i> accession number
	Tep1	<i>Anopheles arabiensis</i> / ACG68562.1	5.00E-11	cyt	HE859562
	Viral A-type inclusion protein	<i>Trichomonas vaginalis</i> G3 / XP_001319569.1	1.00E-06	cyt	HE859685
	Yip1 domain-containing protein	<i>Dictyostelium discoideum</i> / XP_639001	3.00E-31	n.d.	HE859496

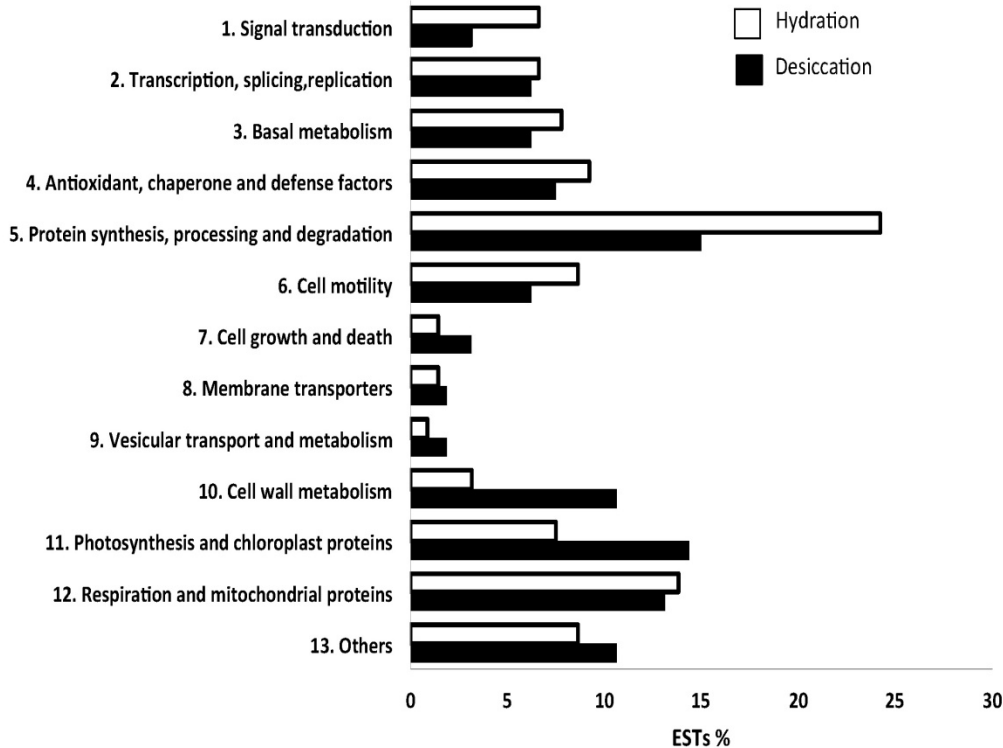


Figure 1. Functional categorization of ESTs obtained from hydration and desiccation *P. columbina* libraries. Percentage values indicate the number of ESTs grouped in each functional group in relation to the total number of sequences obtained in each library.

remodeling is also ATP-dependent (Luo & Dean, 1999). In the case of *P. columbina* it seems reasonable to hypothesize the occurrence of a higher energy production by the mitochondrial system during hydration, as many metabolic reactions are necessary to maintain a homeostatic redox state and, as a result, a healthy physiological condition during the hydration-desiccation cycle. Moreover, the decline in ATP production resulting from a general metabolic slow down during desiccation could be beneficial in preventing ROS production triggered by the electronic alterations induced by low water potential. In vascular plants under water stress, ROS production has been detected in apoplast, xylem vessels, chloroplasts and mitochondria (Mittler *et al.*, 2004; Toldi *et al.*, 2009). Therefore, it is possible that desiccation in *P.*

columbina induces ROS production in the organelles, a hypothesis that needs to be experimentally demonstrated. Additionally, the mitochondrial metabolism should be measured in order to demonstrate a decay-activation sequence of this system during the desiccation-hydration cycle.

In the context of ROS attenuation by the antioxidant system during the hydration-desiccation cycle, several antioxidant enzymes should be expressed. In fact, a peroxiredoxin (PRX) typical 2-Cys, an arachidonate 5-lipoxygenase, a glutathione S-transferase, and several cytochrome P450 and HSPs (heat shock proteins, 27, 40, 70, 80, 90 types) were detected during hydration. Moreover, during desiccation, enzymes such as thioredoxin (TRX), catalase and low variants of HSPs (70 and 90, gene sequences different

from hydrated fronds) were expressed. PRXs are involved in detoxification of hydrogen peroxide, alkylhydroperoxides and peroxinitrites (Hall *et al.*, 2009). These enzymes react at low peroxide concentrations and may become inactive at higher concentrations. In plants, PRX transcripts increase in response to different abiotic stresses such as salinity, drought, and heavy metals (Wood *et al.*, 2003; Dietz, 2011). Their expression in algae has been poorly studied, although some studies indicate they are regulated by light, oxygen, copper, desiccation and redox state (Goyer *et al.*, 2002; Contreras-Porcia *et al.*, 2011a, 2001b; Lovazzano *et al.*, 2013; see section 3 in Contreras-Porcia & López-Cristoffanini, 2012). In vascular plants, several PRXs have been described based on their catalytic mechanisms and subcellular localization (Baier & Dietz, 1997). In this study, PRX expresses mainly when fronds are hydrated, is localized exclusively in the chloroplast, and its reduction is TRX-dependent. The chloroplast TRX activity was also recorded during desiccation (Table 2). Therefore, it seems likely that *P. columbina* PRX play an important role in buffering oxidative stress and in post-desiccation detoxification of lipoperoxides in the chloroplast. However, other attenuation systems might also be operating in both environmental conditions in order to normalize the redox state imbalance. For example, a chlorophycean ferredoxin was identified during desiccation (Table 2). This is a small protein that plays a key role in electron distribution in the chloroplast (Schürmann & Buchanan, 2008) by regulating the chloroplast metabolic network through the TRX system, and contributes directly to ascorbate antioxidant protection (*i.e.*, antioxidant compound and ascorbate peroxidase substrate) and PRX regeneration (Ceccoli *et al.*, 2011; Dietz, 2011).

Another enzyme detected in hydrated fronds was a lipoyxygenase (LOX, Table 1), a dioxygenase which peroxidates polyunsaturated fatty acids (Gigon *et al.*, 2004). Lipid molecules produced by lipid degrading enzymes, such as oxylipins, can act as secondary messengers of stress-response signal transduction pathways (Blée, 2002; Velloso *et al.*, 2007). However, a hyper-stimulation of lipoyxygenase activity could induce an accumulation of lipope-roxides, which leads to cell damage and organelle dysfunction. For example, Contreras *et al.* (2009) demonstrated that hyper-activity of LOX led to an over-production of lipoperoxides, and at the end, to cell death in sensitive species under copper-induced oxidative stress. The particular expression of this enzyme during hydration could explain the exceptional control of the lipid peroxidation in *P. columbina* (Contreras-Porcia *et al.*, 2011a) in comparison with several other algae (Flores-Molina *et al.*, unpublished data). Moreover, the

involvement of PRX during the hydration-desiccation cycle may additionally explain the effective ROS and lipoperoxide attenuation in this species.

Several cytochrome P450 variants were found in *P. columbina* under natural conditions of hydration and desiccation. These enzyme variants are present in all living species and catalyze the oxygenation of a high variety of substrates (Anzenbacher & Anzenbacherová, 2003). It has been demonstrated that P450s are induced by abiotic and biotic stress (Narusaka *et al.*, 2004; Stolf-Moreira *et al.*, 2011). Some P450s have been identified as ABA 8'hydroxylase that degrades ABA (abscidic acid) during the hydration-desiccation cycle (Kushiro *et al.*, 2004; Shinozaki & Yamaguchi-Shinozaki, 2007). In vascular plants, ABA is over-produced during desiccation (as in *P. columbina*, Guajardo *et al. pers. comm.*), causes stomatal closure, and induces stress related genes. However, via the ABA 8'hydroxylase-P450, ABA concentration is reduced to basal levels during the transition from desiccation to hydration (Kushiro *et al.*, 2004). ABA has been recently identified in several algal species (*i.e.*, Tarakhovskaya *et al.*, 2007; Yokoya *et al.*, 2010), although its role in regulating the expression of genes associated with tolerance to abiotic/biotic stress has not being explored. In *P. columbina* the functional role of P450 could open new avenues to learn on tolerance pathways involved in managing environmental stressors, such as the ABA involvement in transcription of regulatory networks of desiccation stress signals and gene expression. Moreover, ABA in *P. columbina* could be involved in the up-regulation of several compounds like sugar, prolines and polyamines (*e.g.*, putrescine, spermidine and spermine), which are known to increase their expression under water stress in vascular plants and algae (Guill & Tuteja, 2010; Alcázar *et al.*, 2011; Kumar *et al.*, 2011). Thus, it is also possible to hypothesize regarding the participation of the ABA-independent pathways in the regulatory response to dehydration stress. Indeed, a Clp-protease was identified during desiccation (Table 2), and the *clp* gene not only was induced by dehydration, but was also up-regulated during natural senescence (Nakashima *et al.*, 1997). Analysis of the *clp* gene in transgenic plants indicates that the *clp* promoter contains cis-acting element(s) involved not only in ABA independent stress-responsive gene expression but also in senescence-activated gene expression (Simpson *et al.*, 2003). Thus, several tolerance pathways, previously unknown in algae and other organisms, could be synergistically activated under particular environmental stress conditions.

Proteases are indispensable for the normal functioning of cells and tissues in all living organisms. However, their activities need to be correctly regulated (Habib & Fazili, 2007). During the hydration-desiccation cycle in *P. columbina*, several peptidases were identified (Tables 1 and 2). Even though the expression of these proteins is required for protein metabolism, an over-expression can be potentially harmful. During hydration, a metallocarboxypeptidase inhibitor (CPI) was identified, that forms a less active or fully inactive enzyme. In vascular plants, CPIs are activated by metals, mechanical wounds or insect injury (Villanueva *et al.*, 1998; Habib & Fazili, 2007; Harada *et al.*, 2010), and its expression is ABA-regulated (Villanueva *et al.*, 1998). Thus, this type of protease in *P. columbina* could be part of the ABA genes that are regulated during the hydration-desiccation cycle needed to maintain cellular integrity under water deficiency.

ABC (ATP binding cassette) transporter sequences were identified in desiccated fronds. These transporters are involved in translocation of a wide variety of compounds across cell membranes, including ions, carbohydrates, lipids, xenobiotics, drugs, and heavy metals (Ehrmann *et al.*, 1998; Sipos & Kuchler, 2006; Contreras *et al.*, 2010). These transporters have been reported during metal tolerance in vascular plants, carrying metal complexes from cytosol to the vacuole (Clemens, 2001). Also, in humans they appear associated with the protection of placental tissue by preventing cellular accumulation of cytotoxic compounds (Aye & Keelan, 2013). The participation of these transporters in desiccated *P. columbina* was unexpected, despite information which recently demonstrated that tolerance to desiccation in the free-living soil bacterium *Rhizobium leguminosarum* was associated with ABC-transporter activity (Vanderlinde *et al.*, 2010). In the bacterium, a mutation in the ATP-binding component of a previously uncharacterized ATP transporter (Young *et al.*, 2006) decreased the tolerance to desiccation due to low exopolysaccharides levels in the cell wall envelope of the mutant. That study demonstrated the crucial role of polysaccharides and their transport in organisms tolerant to desiccation. Therefore, our results suggest, for the first time in algae, the involvement of ABC-transporters in desiccation tolerance, possibly through cell wall stabilization during desiccation stress.

Several HSP were identified during hydration and desiccation stress. These proteins are recognized in prokaryotes and eukaryotes during responses to different physiological and environmental stress conditions (Feder & Hofmann, 1999). HSPs induction

leads to a state of resistance for subsequent stress in the cell by preventing protein aggregation (Feder & Hofmann, 1999) and suppressing apoptosis (*e.g.*, HSP90 see Beere *et al.*, 2000 and Ravagnan *et al.*, 2001). HSP70 blocks apoptosis by binding apoptosis activating factor-1 (Apaf-1), thereby preventing the formation of the apoptosome complex (Ravagnan *et al.*, 2001). In fish hepatocytes, HSPs induction is involved in stress tolerance by modulating the action of key proteins and kinases in the signal transduction pathways (Padmini & Usha-Rani, 2011). In the seaweeds *Fucus serratus* and *F. vesiculosus*, a small HSP has also been recorded in response to abiotic stress, but its specific role remains undetermined (Pearson *et al.*, 2010).

During desiccation sequences of cell-wall hydrolases, glycosyl transferases, and chitin deacetylases (CDA) —all involved in cell wall metabolism— were identified. CDA, a type of carbohydrate esterase, hydrolyzes the acetamide group in the *N*-acetylglucosamine polymers derived from glucose (*e.g.*, chitin), and promotes the formation of glucosamine units (*e.g.*, chitosan). The substrates for this enzyme come from the carbon (fructose 6-phosphate) and nitrogen (glutamine) metabolism (Ghormade *et al.*, 2010). CDA was first discovered in extracts of the fungus *Mucor rouxii* (Araki & Ito, 1975). It was further reported associated with cell wall synthesis. CDA has also been reported in association with spore formation in yeast and attack-defense systems in plant-pathogen interactions. In *P. columbina* an over-activation of this enzyme is likely to occur, due to the induction, under desiccation stress, of a fructose-1,6-phosphatase (Table 2) that can over-produce fructose 6-phosphate, an important precursor of the CDA substrate. Thus, the potential induction of CDA in desiccated *P. columbina* may help in remodeling and maintaining cell wall integrity during growth, survival, and pathogenesis.

CONCLUSIONS

Despite their crucial ecological role as primary producers, molecular information on stress responses in intertidal macroalgae remains limited. *Pyropia columbina*, such as others *Porphyra* and *Pyropia* species, is a good model for unravelling some of the biological and molecular responses associated with desiccation and other environmental conditions that may cause oxidative stress. In this context, two subtracted EST libraries were constructed in order to understand the metabolic pathways active during the hydration-desiccation cycle in this species. Results

showed that a significant portion of the transcripts had no known homologues in algae or other organisms as far as sequence data were available. These sequences are interesting since they could represent genes unique to this species. On the other hand, several genes/proteins not previously described in algae were differentially expressed in both environmental conditions. This information contributes to a better understanding the molecular mechanisms involved in tolerance to desiccation. However, a confirmation of the expression profiles of the reported genes by qPCR is needed to characterize, for example, temporality of the gene expression profile during the hydration-desiccation cycle. Additionally, our study provides for the first time, a set of candidate genes for further examination of the physiological responses to other environmental stressors. This genetic background will broaden our understanding on physiological differences may contribute, or even determine, the ecological features of macroalgae inhabiting the intertidal rocky zone.

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